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## (57) Abstract

A purified preparation of a peptide comprising an amino acid sequence identical to that of a segment of a naturally-occurring human protein, said segment being of 10 to 30 residues in length, inclusive, wherein said peptide binds to a human major histocompatibility complex (MHC) class II allotype.

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## IMMUNOMODULATORY PEPTIDES

The field of the invention is major histocompatibility complex (MHC) antigens.

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## Background of the Invention

Major histocompatibility complex (MHC) class II antigens are cell surface receptors that orchestrate all specific immune responses in vertebrates. Humans possess three distinct MHC class II isotypes: DR, for which approximately 70 different allotypes are known; DQ, for which 33 different allotypes are known; and DP, for which 47 different allotypes are known. Each individual bears two to four DR alleles, two DQ alleles, and two DP alleles.

MHC receptors (both class I and class II) 15 participate in the obligate first step of immune recognition by binding small protein fragments (peptides) derived from pathogens or other non-host sources, and presenting these peptides to the regulatory cells (T 20 cells) of the immune system. In the absence of MHC presentation, T cells are incapable of recognizing pathogenic material. Cells that express MHC class II receptors are termed antigen presenting cells (APC). APCs ingest pathogenic organisms and other foreign 25 materials by enveloping them in endosomic vesicles, then subjecting them to enzymatic and chemical degradation. Foreign proteins which are ingested by APCs are partially degraded or "processed" to yield a mixture of peptides, some of which are bound by MHC class II molecules that 30 are en route to the surface. Once on the cell surface, MHC-bound peptides are available for T cell recognition.

MHC class II antigens are expressed on the surface of APCs as a trimolecular complex composed of an  $\alpha$  chain, a  $\beta$  chain, and a processed peptide. Like most polypeptides that are expressed on the cell surface, both

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 $\alpha$  and  $\beta$  chains contain short signal sequences at their NH2 termini which target them to the endoplasmic reticulum (ER). Within the ER the class II  $\alpha/\beta$  chain complex associates with an additional protein termed the 5 invariant chain (Ii). Association with Ii is proposed to block the premature acquisition of peptides (by blocking the peptide binding cleft of the MHC heterodimer), promote stable  $\alpha/\beta$  interaction, and direct subsequent intracellular trafficking of the complex to endosomal 10 vesicles. In the endosomes, Ii is removed by a process involving proteolysis; this exposes the peptide binding cleft, thus allowing peptides present in the endosome to bind to the MHC molecule. The class II/ peptide complex is transported from the endosomes to the cell surface 15 where it becomes accessible to T-cell recognition and subsequent activation of immune responses. Class II MHC molecules bind not only to peptides derived from exogenous (ingested) proteins, but also to those produced by degradation of endogenous (self) proteins. The amount 20 of each species of peptide which binds class II is determined by its local concentration and its relative binding affinity for the given class II binding groove, with the various allotypes displaying different peptidebinding specificities.

Early during fetal development, the mammalian immune system is "tolerized", or taught not to react, to self-peptides. The stability and maintenance of this system is critical for ensuring that an animal does not generate an immune response against self. A breakdown of this system gives rise to autoimmune conditions such as diabetes, rheumatoid arthritis and multiple sclerosis. Current technologies intended to manipulate the immune system into reestablishing proper nonresponsiveness include protocols involving the intravenous delivery of synthetic, high affinity binding peptides as blocking peptides.

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Vaccination can generate protective immunity against a pathogenic organism by stimulating an antibodymediated and/or a T cell-mediated response. Most of the current vaccination strategies still use relatively crude preparations, such as attenuated or inactivated viruses. These vaccines often generate both antibody— and cell-mediated immunity, and do not allow one to modulate the type of immune response generated. Moreover, in many diseases the generation of the wrong type of response can result in an exacerbated disease state.

## Summary of the Invention

In the work disclosed herein, naturally processed peptides bound to six of the some 70 known human MHC class II DR allotypes (HLA-DR1, HLA-DR2, HLA-DR3, HLA-15 DR4, HLA-DR7, and HLA-DR8) have been characterized. These peptides were found to be predominantly derived from self proteins rather than foreign proteins. Several self peptide families have been identified with the unexpected property of degenerate binding: that is, a 20 given self-peptide will bind to a number of HLA-DR allotypes. This observation runs counter to the widelyaccepted view of MHC class II function, which dictates that each allotype binds a different set of peptides. Furthermore, many if not all of the self-peptides 25 disclosed herein bind to the class II molecules with relatively high affinity. These three characteristics--(1) self rather than foreign, (2) degeneracy, and (3) high affinity binding--suggest a novel means for therapeutic intervention in disease conditions 30 characterized by autoreactivity, such as Type I diabetes, rheumatoid arthritis, and multiple sclerosis. In addition, such therapy could be used to reduce transplant rejection.

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In the therapeutic methods of the invention, short peptides modelled on the high-affinity immunomodulating self peptides of the invention (which preferably are nonallelically restricted) are introduced into the APCs 5 of a patient. Tissue typing to determine the particular class II alleles expressed by the patient may be unnecessary, as the peptides of the invention are bound by multiple class II isotypes. It may be useful to employ a "cocktail" of peptides, where complete 10 degeneracy is lacking for individual peptides, i.e., where peptides binds to fewer than all allotypes; the cocktail provides overlapping binding specificity. Once in the APC, a peptide binds to the class II molecules with high affinity, thereby blocking the binding of 15 immunogenic peptides which are responsible for the immune reaction characteristic of the disease condition. Because the blocking peptides of the invention are self peptides with the exact carboxy and amino termini tolerized during ontogeny, they are immunologically inert 20 and will not induce an immune response which may complicate treatment using non-self blocking peptides.

into APCs directly, e.g., by intravenous injection of a sclution containing one or more of the peptides. 
25 Alternatively, the APCs may be provided with a means of synthesizing large quantities of the blocking peptides intracellularly. Recombinant genes that encode ER and/or endosomal targeting signals fused to blocking peptide sequences are linked to appropriate expression control sequences and introduced into APCs. Once in the cell, these genes direct the expression of the hybrid peptides. Peptides targeted to the ER will bind class II  $\alpha$  and  $\beta$  chains as they are translated and assembled into heterodimers. The presence of high affinity binding

35 peptides within the ER will prevent association of the

The peptides of the invention may be introduced

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 $\alpha/\beta$  complex with invariant chain, and thus interfere with intracellular trafficking. The class II molecule/ blocking peptide complex may subsequently be expressed on the cell surface, but would not elicit an immune response 5 since T cells are tolerized to this complex early in development. The use of peptides tagged with ER retention signals may also prevent the peptide-complexed class II molecules from leaving the ER. Alternatively, the recombinant peptide may be tagged with an endosomal 10 targeting signal which directs it to the endosomal compartment after synthesis, thereby also skewing the ratio of endogenously-processed peptide to blocking peptide in the endosome and favoring binding of the high affinity blocking peptide to any class II molecules which 15 did not bind it in the ER. It may be advantageous, for any individual patient, to employ one or more ER-directed peptides in combination with one or more endosomedirected peptide, so that  $\alpha-\beta$  complexes which are not filled in the ER with peptides of the invention are then 20 blocked in the endocytic pathway. The end result again is cell surface expression of a non-immunogenic class II/peptide complex.

The use of a class II nonrestricted high affinity binding peptide coupled to an intracellular delivery

25 system permits the specific down-regulation of class II restricted immune responses without invoking the pleiotropic adverse reactions associated with the current pharmacological strategies. Successful application of these technologies will constitute a significant advance towards the treatment of autoimmune disease and prevention of transplant rejection.

The intracellular delivery system of the invention can also be utilized in a novel method of vaccination of an animal, e.g., a human patient or a commercially significant mammal such as a cow which is susceptible to

diseases such as hoof and mouth disease. Such a system can be tailored to generate the type of immune response required in a given situation by adjustments in the following: (a) peptide specificity for class I or class 5 II MHC; (b) peptide/protein length and/or sequence, and (c) using specific tags for organelle targeting. system of the invention ensures that peptides are produced only within cells, and are not present outside the cells where they could stimulate antibody production 10 by contact with B cells. This limits the immune response generated by such a vaccine to T cell-mediated immunity, thereby preventing either an inappropriate or potentially deleterious response as might be observed with standard vaccines targeting the organisms which cause, for 15 example, HIV, malaria, leprosy, and leishmaniasis. Furthermore, this exclusively T cell-mediated immune response can be class I or class II-based, or both, depending upon the length and character of the immunogenic peptides: MHC class I molecules are known to 20 bind preferentially to peptides 8 to 10 residues in length, while class II molecules bind with high affinity to peptides that range from 12 to 25 residues long.

Immunization and therapy according to the invention can employ a purified preparation of a peptide 25 of the invention, i.e., a peptide which includes an amino acid sequence identical to that of a segment of a naturally-occurring human protein (i.e., a "self protein"), such segment being of 10 to 30 residues in length, wherein the peptide binds to a human MHC class II allotype, and preferably binds to at least two distinct MHC class II allotypes (e.g., any of the approximately 70 known DR allotypes, approximately 47 known DP allotypes, or approximately 33 known DQ allotypes). The portion of the peptide corresponding to the self protein segment is 35 herein termed a "self peptide". By "purified

preparation" is meant a preparation at least 50% (by weight) of the polypeptide constituents of which consists of the peptide of the invention. In preferred embodiments, the peptide of the invention constitutes at 5 least 60% (more preferably at least 80%) of the purified preparation. The naturally-occurring human protein is preferably HLA-A2 (as broadly defined below), HLA-A29, HLA-Bw62, HLA-C, HLA-DR $\alpha$ , HLA-DR $\beta$ , invariant chain (Ii), Ig kappa chain C region, Ig heavy chain, Na<sup>+</sup>/K<sup>+</sup> ATPase, 10 transferrin, transferrin receptor, calcitonin receptor, carboxypeptidase E, MET kinase-related transforming protein, guanylate-binding protein, mannose-binding protein, apolipoprotein B-100, cathepsin C, cathepsin S, metalloproteinase inhibitor 1 precursor, or heat shock 15 cognate 71 kD protein; it may be an MHC class I or II antigen protein or any other human protein which occurs at the cell surface of APCs. The self peptide preferably conforms to the following motif: at a first reference position (I) at or within 12 residues of the amino 20 terminal residue of the segment, a positively charged residue (i.e., Lys, Arg, or His) or a large hydrophobic residue (i.e., Phe, Trp, Leu, Ile, Met, Tyr, or Pro; and at position I+5, a hydrogen bond donor residue (i.e., Tyr, Asn, Gln, Cys, Asp, Glu, Arg, Ser, Trp, or Thr). 25 addition, the peptide may also be characterized as having, at positions I+9, I+1, and/or I-1, a hydrophobic residue (i.e., Phe, Trp, Leu, Ile, Met, Pro, Ala, Val, or Tyr) (+ denotes positions to the right, or toward the carboxy terminus, 30 and - denotes positions to the left, or toward the amino

and - denotes positions to the left, or toward the amino terminus.) A typical peptide of the invention will include a sequence corresponding to residues 31-40 (i.e., TQFVRFDSDA) or residues 106-115 (i.e., DWRFLRGYHQ) of HLA-A2, or residues 107-116 (i.e., RMATPLLMQA) of Ii, or

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a sequence essentially identical to any one of the sequences set forth in Tables 1-10 below.

The therapeutic and immunization methods of the invention can also employ a nucleic acid molecule (RNA or 5 DNA) encoding a peptide of the invention, but encoding less than all of the entire sequence of the self protein. The nucleic acid preferably encodes no substantial portion of the self protein other than the specified self peptide which binds to a MHC class II molecule, although 10 it may optionally include a signal peptide or other trafficking sequence which was derived from the self protein (or from another protein). A trafficking sequence is an amino acid sequence which functions to control intracellular trafficking (directed movement from 15 organelle to organelle or to the cell surface) of a polypeptide to which it is attached. Such trafficking sequences might traffic the polypeptide to ER, a lysosome, or an endosome, and include signal peptides (the amino terminal sequences which direct proteins into 20 the ER during translation), ER retention peptides such as KDEL; and lysosome-targeting peptides such as KFERQ, QREFK, and other pentapeptides having Q flanked on one side by four residues selected from K, R, D, E, F, I, V, and L. An example of a signal peptide that is useful in 25 the invention is a signal peptide substantially identical to that of an MHC subunit such as class II  $\alpha$  or  $\beta$ ; e.g., the signal peptide of MHC class II  $\alpha$  is contained in the sequence MAISGVPVLGFFIIAVLMSAQESWA. The signal peptide encoded by the nucleic acid of the invention may include 30 only a portion (e.g., at least ten amino acid residues) of the specified 25 residue sequence, provided that portion is sufficient to cause trafficking of the polypeptide to the ER. In preferred embodiments, the nucleic acid of the invention encodes a second self 35 peptide and a second trafficking sequence (which may be

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identical to or different than the first self peptide and first trafficking sequence), and it may encode additional self peptides and trafficking sequences as well. still another variation on this aspect of the invention, 5 the self peptide sequence (or a plurality of self peptide sequences arranged in tandem) is linked by a peptide bond to a substantially intact Ii polypeptide, which then carries the self peptide sequence along as it traffics the class II molecule from ER to endosome.

The nucleic acid of the invention may also contain expression control sequences (defined as transcription and translation start signals, promoters, and enhancers which permit and/or optimize expression of the coding sequence with which they are associated) and/or genomic 15 nucleic acid of a phage or a virus, such as an attenuated or non-replicative, non-virulent form of vaccinia virus, adenovirus, Epstein-Barr virus, or a retrovirus.

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The peptides and nucleic acids of the invention may be prepared for therapeutic use by suspending them 20 directly in a pharmaceutically acceptable carrier, or by encapsulating them in liposomes, immune-stimulating complexes (ISCOMS), or the like. Such preparations are useful for inhibiting an immune response in a human patient, by contacting a plurality of the patient's APCs 25 with the therapeutic preparation and thereby introducing the peptide or nucleic acid into the APCs.

Also within the invention is a cell (e.g., a tissue culture cell or a cell, such as a B cell or APC, within a human) containing the nucleic acid molecule of 30 the invention. A cultured cell containing the nucleic acid of the invention may be used to manufacture the peptide of the invention, in a method which involves culturing the cell under conditions permitting expression of the peptide from the nucleic acid molecule.

Disclosed herein is a method of identifying a nonallelically restricted immunomodulating peptide, which method includes the steps of:

- (a) fractionating a mixture of peptides eluted 5 from a first MHC class II allotype;
  - (b) identifying a self peptide from this mixture; and
- (c) testing whether the self peptide binds to a second MHC class II allotype, such binding being an 10 indication that the self peptide is a nonallelically restricted immunomodulating peptide.

In further embodiments, the invention includes a method of identifying a potential immunomodulating peptide, in a method including the steps of:

- 15 (a) providing a cell expressing MHC class II molecules on its surface;
  - (b) introducing into the cell a nucleic acid encoding a candidate peptide; and
- (c) determining whether the proportion of 20 class II molecules which are bound to the candidate peptide is increased in the presence of the nucleic acid compared to the proportion bound in the absence of the nucleic acid, such an increase being an indication that the candidate peptide is a potential immunomodulating 25 peptide.

Also within the invention is a method of identifying a potential immunomodulating peptide, which method includes the steps of:

(a) providing a cell expressing MHC class II30 molecules on its surface;

. .

- (b) introducing into the cell a nucleic acid encoding a candidate peptide; and
- (c) determining whether the level of MHC class II molecules on the surface of the cell is decreased in the 35 presence of the nucleic acid compared to the level of MHC

class II molecules in the absence of the nucleic acid, such a decrease being an indication that the candidate peptide is a potential immunomodulating peptide.

Also included in the invention is a method of identifying a nonallelically restricted immunostimulating peptide, which method includes the steps of:

- (a) providing a cell bearing a first MHC class I or class II allotype, such cell being infected with a pathogen (e.g., an infective agent which causes human or animal disease, such as human immunodeficiency virus (HIV), hepatitis B virus, measles virus, rubella virus, influenza virus, rabies virus, Corynebacterium diphtheriae, Bordetella pertussis, Plasmodium spp., Schistosoma spp., Leishmania spp., Trypanasoma spp., or Mycobacterium lepre);
  - (b) eluting a mixture of peptides bound to the cell's first MHC allotype;
- (c) identifying a candidate peptide from the mixture, such candidate peptide being a fragment of a 20 protein from the pathogen; and
- (d) testing whether the candidate peptide binds to a second MHC allotype, such binding being an indication that the candidate peptide is a nonallelically restricted immunostimulating peptide. A nucleic acid encoding such an immunogenic fragment of a protein of a pathogen can be used in a method of inducing an immune response in a human patient, which method involves introducing the nucleic acid into an APC of the patient.

The therapeutic methods of the invention solve

30 certain problems associated with prior art methods
involving intravenous injection of synthetic peptides:

(1) because of allelic specificity, a peptide capable of
binding with high affinity to all, or even most, of the
different class II allotypes expressed within the general

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population had not previously been identified; (2) the half-lives of peptides delivered intravenously are generally very low, necessitating repeated administration with the associated high level of inconvenience and cost; (3) this type of delivery approach requires that the blocking peptide displace the naturally-occurring peptide occupying the binding cleft of a class II molecule while the latter is on the cell surface, which is now believed to be a very inefficient process; and (4) if the blocking peptide utilized is itself immunogenic, it may promote deleterious immune responses in some patients.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

#### Detailed Description

The drawings are first briefly described.

Drawings

Figs. 1a-f are chromatographic analyses of the peptide pools extracted from papain digested HLA-DR1, 20 DR2, DR3, DR4, DR7, and DR8, respectively, illustrating the peptide repertoire of each HLA-DR as detected by UV absorbance. The UV absorbance for both 210 nm and 277 nm is shown at a full scale absorbance of 500 mAU with a retention window between 16 minutes and 90 minutes (each 25 mark represents 2 minutes).

Fig. 2 is a representative mass spectrometric analysis of the size distribution of isolated HLA-DR1 bound peptides. The determined peptide masses in groups of 100 mass units were plotted against the number of isolated peptides identified by mass spectrometry. Peptide length was calculated by dividing the experimental mass by an average amino acid mass of 118 daltons.

Fig. 3 is a representation of two minigenes of the 35 invention, in which the  $HLA-DR\alpha$  chain leader peptide is

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linked to the amino terminus of a 15-residue (A) or 24-residue (B) blocking peptide fragment of human invariant chain Ii.

#### Experimental Data

#### 5 METHODS

#### I. Purification of HLA-DR antigens.

HLA-DR molecules were purified from homozygous, Epstein-Barr virus-transformed, human B lymphoblastoid lines: DR1 from LG-2 cells, DR2 from MST cells, DR3 from 10 WT20 cells, DR4 from Priess cells, DR7 from Mann cells, and DR8 from 23.1 cells. All of these cell lines are publicly available. Cell growth, harvest conditions and protein purification were as previously described (Gorga, J. et al., 1991). Briefly, 200 grams of each cell type 15 was resuspended in 10mM Tris-HCl, 1mM dithiothreitol (DTT), 0.1mM phenylmethylsulfonylflouride (PMSF), pH 8.0, and lysed in a Thomas homogenizer. The nuclei were removed by centrifugation at 4000xg for 5 min and the pellets washed and repelleted until the supernatants were 20 clear. All the supernatants were pooled and the membrane fraction harvested by centrifugation at 175,000xg for 40 min. The pellets were then resuspended in 10 mM Tris-HCl, 1mM DTT, 1mM PMSF, 4% NP-40. The unsolubilized membrane material was removed by centrifugation at 25 175,000xg for 2 hours, and the NP-40 soluble supernatant fraction used in immunoaffinity purification.

Detergent soluble HLA-DR was bound to a LB3.1protein A sepharose column (Gorga et al., <u>id</u>) and eluted
with 100 mM glycine, pH 11.5. Following elution, the

30 sample was immediately neutralized by the addition of
Tris-HCl and then dialyzed against 10mM Tris-HCl, 0.1%
deoxycholic acid (DOC). The LB3.1 monoclonal antibody
recognizes a conformational determinant present on the

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nonpolymorphic HLA-DR $\alpha$  chain, and thus recognizes all allotypes of HLA-DR.

The transmembrane domain of the DR molecules was removed by papain digestion, and the resulting water5 soluble molecule further purified by gel filtration chromatography on an S-200 column equilibrated in 10mM Tris-HCl, pH 8.0. The purified DR samples were concentrated by ultrafiltration, yield determined by BCA assay, and analyzed by SDS polyacrylamide gel
10 electrophoresis.

# II. Extraction and fractionation of bound peptides.

Water-soluble, immunoaffinity-purified class II molecules were further purified by high-performance size exclusion chromatography (SEC), in 25 mM N-morpholino 15 ethane sulfonic acid (MES) pH 6.5 and a flowrate of 1 ml/min., to remove any residual small molecular weight contaminants. Next, Centricon microconcentrators (molecular weight cutoff 10,000 daltons) (Amicon Corp.) were sequentially washed using SEC buffer and 10% acetic 20 acid prior to spin-concentration of the protein sample (final volume between 100-200  $\mu$ l). Peptide pools were extracted from chosen class II alleles by the addition of 1 ml of 10% acetic acid for 15 minutes at 70°C. These conditions are sufficient to free bound peptide from 25 class II molecules, yet mild enough to avoid peptide degradation. The peptide pool was separated from the class II molecule after centrifugation through the Centricon concentrator, with the flow-through containing the previously bound peptides.

The collected acid-extracted peptide pool was concentrated in a Savant Speed-Vac to a volume of 50 μl prior to HPLC separation. Peptides were separated on a microbore C-18 reversed-phase chromatography (RPC) column (Vydac) utilizing the following non-linear gradient protocol at a constant flowrate of 0.15 ml/min.: 0-63

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min. 5%-33% buffer B; 63-95 min. 33%-60% buffer B; 95-105 min 60%-80% buffer B, where buffer A was 0.06% trifluoroacetic acid/water and buffer B was 0.055% trifluoroacetic acid/acetonitrile. Chromatographic 5 analysis was monitored at multiple UV wavelengths (210, 254, 277, and 292 nm) simultaneously, permitting spectrophotometric evaluation prior to mass and sequence analyses. Shown in Fig.1 are chromatograms for each of the six DR peptide pools analyzed. Collected fractions were subsequently analyzed by mass spectrometry and Edman sequencing.

## III. Analysis of peptides.

The spectrophotometric evaluation of the peptides during RPC provides valuable information regarding amino 15 acid composition (contribution of aromatic amino acids) and is used as a screening method for subsequent characterization. Appropriate fractions collected during the RPC separation were next analyzed using a Finnegan-MAT LaserMat matrix-assisted laser-desorption mass 20 spectrometer (MALD-MS) to determine the individual mass values for the predominant peptides. Between 1%-4% of the collected fraction was mixed with matrix (1 $\mu$ l  $\alpha$ -Cyano-4-hydroxycinnamic acid) to achieve mass determination of extracted peptides. The result of this 25 analysis for HLA-DR1 is shown in Fig. 2. Next, chosen peptide samples were sequenced by automated Edman degradation microsequencing using an ABI 477A protein sequencer (Applied Biosystems) with carboxy-terminal verification provided by mass spectral analysis using the 30 Finnigan-MAT TSQ 700 triple quadruple mass spectrometer equipped with an electro-spray ion source. This parallel analysis ensures complete identity of peptide composition and sequence. Peptide alignment with protein sequences stored in the SWISS-PROT database was performed using the

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FASTA computer database search program. Set forth in Tables 1-10 are the results of this sequence analysis for each of the DR molecules studied.

#### RESULTS

#### 5 I. HLA-DR1.

The HLA-DR1 used in this study was papain solubilized to enable the material to be used both for crystallographic and bound peptide analyses. peptides bound to DR1 were acid extracted and 10 fractionated using RPC (Fig. 1). The absence of any detectable peptidic material following a second extraction/RPC separation verified quantitative peptide extraction. Amino acid analysis (ABI 420A/130A derivatizer/HPLC) of extracted peptide pools demonstrated 15 a 70-80% yield, assuming total occupancy of purified DR1 with a molar equivalent of bound peptides corresponding to the size distribution determined by mass spectrometry (see Fig. 2). The RPC profiles obtained from DR1 extractions of multiple independent preparations were 20 reproducible. Furthermore, profiles from either detergent-soluble or papain-solubilized DR1 were equivalent. To confirm that the peptides were in fact identical in detergent-soluble and papain-digested DR1, mass spectrometry and Edman sequencing analyses were 25 performed and revealed identical masses and sequences for analogous fractions from the two preparations.

Matrix-assisted laser desorption mass spectrometry (MALD-MS) was used to identify 111 species of unique mass contained within the eluted peptide pool of DR1 with an average size of 18 and a mode of 15 residues (Fig. 2). Over 500 additional mass species present within the molecular weight range of 13-25 residues were detected; however, the signal was not sufficient to assign individual masses with confidence. Multiple species of

varying mass were detected in fractions corresponding to single RPC peaks indicating co-elution of peptides. To characterize these peptides further, samples were analyzed in parallel on a triple quadruple mass

5 spectrometer equipped with an electrospray ion source (ESI-MS) and by automated Edman degradation microsequencing (Lane et al., J. Prot. Chem. 10:151-160 (1991)). Combining these two techniques permits crucial verification of both the N- and C-terminal amino acids of peptides contained in single fractions. The sequence and mass data acquired for twenty peptides isolated from DR1 are listed in Table 1. All the identified peptides aligned with complete identity to regions of proteins stored in the SWISS-PROT database.

Surprisingly, sixteen of the twenty sequenced DR1-bound peptides were 100% identical to regions of the self proteins HLA-A2 and class II-associated invariant chain (Ii), representing at least 26% of the total extracted peptide mass. These isolated peptides varied in length and were truncated at both the N- and C-termini, suggesting that: 1) antigen processing occurs from both ends after binding to DR1, or 2) class II molecules bind antigen from a pool of randomly generated peptides. The yields from the peptide microsequencing indicated that HLA-A2 (Fig. 1) and Ii each represents at least 13% of the total DR1-bound peptides.

An additional surprising finding concerned a peptide which, although bound to HLA-DR and 100% homologous with HLA-A2 peptide, was derived from a cell which does not express HLA-A2 protein. Evidently this peptide is derived from a protein containing a region homologous with a region of HLA-A2 protein. Thus, for purposes of this specification, the term "HLA-A2 protein" is intended to include HLA-A2 protein itself, as well as any naturally occurring protein which contains a ten or

greater amino acid long region of >80% homology with an HLA-DR-binding peptide derived from HLA-A2. An "HLA-A2 peptide" similarly refers to peptides from any HLA-A2 protein, as broadly defined herein.

5 The other four peptides identified in the DR1 studies were derived from two self proteins, transferrin receptor and the Na<sup>+</sup>/K<sup>+</sup> ATPase, and one exogenous protein, bovine serum fetuin (a protein present in the serum used to fortify the medium which bathes the cells). 10 these peptides occupied only 0.3-0.6% of the total DR1 population, significantly less than either the HLA-A2 or the Ii peptides. It is known that class II molecules en route to the cell surface intersect the pathway of incoming endocytic vesicles. Both recycling membrane 15 proteins and endocytosed exogenous protein travel this common pathway. Hence, the HLA-A2, transferrin receptor, Na<sup>+</sup>/K<sup>+</sup> ATPase and bovine fetuin derived peptides would all encounter DR1 in a similar manner. Ii associates with nascent class II molecules in the endoplasmic reticulum 20 (ER) (Jones et al., Mol. Immunol. 16:51-60 (1978)), preventing antigen binding until the class II/Ii complex arrives at an endocytic compartment (Roche and Cresswell, Nature 345:615-618 (1990)), where Ii undergoes proteolysis (Thomas et al., J. Immunol. 140:2670-2675 25 (1988); Roche and Cresswell, Proc. Natl. Acad. Sci. USA 88:3150-3154 (1991)), thus allowing peptide binding to proceed. Presumably, the Ii peptides bound to DR1 were generated at this step.

Synthetic peptides corresponding to five of the
30 peptides reported in Table 1 were made and their relative
binding affinities to DR1 determined. The influenza A
hemagglutinin peptide (HA) 307-319 has been previously
described as a high affinity, HLA-DR1 restricted peptide
(Roche and Cresswell, J. Immunol. 144:1849-1856 (1990);
35 Rothbard et al., Cell 52:515-523 (1988)), and was thus

chosen as the control peptide. "Empty" DR1 purified from insect cells expressing recombinant DR1 cDNA was used in the binding experiments because of its higher binding capacity and 10-fold faster association kinetics than DR1 5 isolated from human cells (Stern and Wiley, Cell 68:465-477 (1992)). All the synthetic peptides were found to compete well (Ki < 100 nM) against the HA peptide (Table At first approximation, the Ii 106-119 peptide had the highest affinity of all the competitor peptides 10 measured, equivalent to that determined for the control HA peptide. In addition to the Ki determinations, these peptides were found to confer resistance to SDS-induced  $\alpha$ - $\beta$  chain dissociation of "empty" DR1 when analyzed by SDS-PAGE, indicative of stable peptide binding (Sadegh-15 Nasseri and Germain, Nature 353:167-170 (1991); Dornmair et al., Cold Spring Harbor Symp. Quant. Biol. 54:409-415 (1989); Springer et al., J. Biol. Chem. 252:6201-6207 (1977)). Neither of the two control peptides,  $\beta_2$ m 52-64 nor Ii 96-110, was able to either confer resistance to 20 SDS-induced chain dissociation of DR1 or compete with HA 307-319 for binding to DR1; both of these peptides lack the putative binding motif reported in this study (see below).

A putative DR1 binding motif based on the sequence alignments of the core epitopes (the minimum length) of certain naturally processed peptides is shown in Table 3. The peptides listed in this table include those determined herein for HLA-DR1, as well as a number of peptides identified by others and known to bind DR1 (reference #6 in this table being O'Sullivan et al., J. Immunol. 145:1799-1808, 1990; reference #17, Roche & Cresswell, J. Immunol. 144:1849-1856, 1990; reference #25, Guttinger et al., Intern. Immunol. 3:899-906, 1991; reference #27, Guttinger et al. EMBO J. 7:2555-2558, 1988; and reference #28, Harris et al., J. Immunol.

148:2169-2174, 1992). The key residues proposed in the motif are as follows: a positively charged group is located at the first position, referred to here as the index position for orientation (I); a hydrogen bond donor is located at I+5; and a hydrophobic residue is at I+9. In addition, a hydrophobic residue is often found at I+1 and/or I-1. Every naturally processed peptide sequenced from DR1 conforms to this motif (with the exception of the HLA-A2 peptide 103-116 that lacks residue I+9).

- 10 Because the putative motif is not placed in a defined position with respect to the first amino acid and because of the irregular length of bound peptides, it is impossible to deduce a motif from sequencing of peptide pools, as was done for class I molecules (Falk et al.,
- 15 Nature 351:290-296 (1991)). The Ii 96-110 peptide, a negative control peptide used in binding experiments, has the I and I+5 motif residues within its sequence, but is missing eight additional amino acids found in Ii 105-118 (Table 3C).
- A sequence comparison of 35 previously described DR1-binding synthetic peptides (O'Sullivan et al., J. Immunol. 145:1799-1808 (1990); Guttinger et al., Intern. Immunol. 3:899-906 (1991); Hill et al., J. Immunol. 147:189-197 (1991); Guttinger et al., EMBO J. 7:2555-2558 (1988); Harris et al., J. Immunol. 148:2169-2174 (1992))
  - also supports this motif. Of the 35 synthetic peptides, 21 (60%) have the precise motif, nine (30%) contain a single shift at either I or I+9, and the remaining five (10%) have a single substitution at I (Table 3B and C).
- 30 Interestingly, in the latter peptides, a positive charge at I is always replaced by a large hydrophobic residue (Table 8C); a pocket has been described in class I molecules that can accommodate this precise substitution (Latron et al., Proc. Natl. Acad. Sci. USA 88:11325-11329
- 35 (1991)). Contributions by the other eight amino acids

within the motif or the length of the peptide have not been fully evaluated and may compensate for shifted/missing residues in those peptides exhibiting binding. Evaluation of the remaining 117 non-DR1 binding 5 peptides cited in those studies (which peptides are not included in Table 3) indicates that 99 (85%) of these peptides do not contain the DR1 motif proposed herein. Of the remaining 18 peptides (15%) that do not bind to DR1 but which do contain the motif, 6 (5%) are known to bind to other DR allotypes; the remaining 12 peptides may have unfavorable interactions at other positions which interfere with binding.

In contrast to the precise N-terminal cleavages observed in the previous study of six peptides bound to 15 the mouse class II antigen termed I-Ab and five bound to mouse I-Eb (Rudensky et al., Nature 3563:622-627 (1991)), the peptides bound to DR1 are heterogeneous at both the N- and C-termini. In contrast to peptides bound to class I molecules, which are predominantly nonamers (Van Bleek 20 and Nathenson, Nature 348:213-216 (1990); Rotzschke et al., Nature 348:252-254 (1990); Jardetzky et al., Nature 353:326-329 (1991); Hunt et al., Science 255:1261-1263 (1992)), class II peptides are larger and display a high degree of heterogeneity both in length and the site of 25 terminal truncation, implying that the mechanisms of processing for class I and class II peptides are substantially different. Furthermore, the present results suggest that class II processing is a stochastic event and that a DR allotype may bind peptides of 30 different lengths from a complex random mixture. The heterogeneity observed may be solely due to protection of bound peptides from further degradation. Thus, class II molecules would play an active role in antigen processing (as previously proposed (Donermeyer and Allen, J. 35 Immunol. 142:1063-1068 (1989)) by protecting the bound

peptides from complete degradation. Alternatively, the predominance of 15mers bound to DR1 (as detected by both the MALD-MS and the yields of sequenced peptides) could be the result of trimming of bound peptides. In any event, the absence of detectable amounts of peptides shorter than 13 and longer than 25 residues suggests that there are length constraints intrinsic either to the mechanism of peptide binding or to antigen processing. The predominance of peptides bound to DR1 that are derived from endogenously synthesized proteins, and particularly MHC-related proteins, may result from the evolution of a mechanism for presentation of self peptides in connection with the generation of self tolerance.

## 15 II. Other HLA-DR molecules.

The sequences of naturally processed peptides eluted from each of DR2, DR3, DR4, DR7 and DR8 are shown in

Tables 4-8, respectively. Table 9 gives sequences of DR1
20 from another cell line which does not have wild-type Ar,
but which has bound A2-like peptides. Table 10 gives
sequences of peptides eluted from DR4 and DR11 molecules
expressed in cells from a human spleen. These data
demonstrate the great prevalence of self peptides bound,
25 compared to exogenous peptides. The data also show that
the A2 and Ii peptides occur repeatedly.

# III. Peptide Delivery

Genetic Constructions.

In order to prepare genetic constructs for <u>in vivo</u>

30 administration of genes encoding immunomodulatory
peptides of the invention, the following procedure is
carried out.

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Overlapping synthetic oligonucleotides were used to generate the leader peptide/blocking peptide minigenes illustrated in Fig. 3 by PCR amplification from human HLA-DRα and invariant chain cDNA templates. These 5 minigenes encode the Ii peptide fragments KMRMATPLLMQALPM (or Ii<sub>15</sub>) and LPKPPKPVSKMRMATPLLMQALPM (or Ii<sub>24</sub>). The resulting constructs were cloned into pGEM-2 (Promega Corp.) to form the plasmids pGEM-2-α-Ii<sub>15</sub> and pGEM-2-α-Ii<sub>24</sub>, with an upstream T7 promoter for use in the in vitro transcription/translation system described below.

For in vivo expression, each mini-gene was subsequently subcloned from the pGEM-2 derivatives into a transfection vector, pHβactin-1-neo (Gunning et al., (1987) P.N.A.S. U.S.A. 84:4831), to form the plasmids pHβactin-α-Ii<sub>15</sub> and pHβactin-α-Ii<sub>24</sub>. The inserted minigenes are thus expressed in vivo from the constitutive/strong human β actin promoter. In addition, the mini-genes were subcloned from the pGEM-2 derivatives into the vaccinia virus recombination vector pSC11 (S. Chakrabarti et al. (1985) Mol. Cell Biol. 5, 3403-3409) to form the plasmids pSC11-α-Ii<sub>15</sub> and pSC11-α-Ii<sub>24</sub>. Following recombination into the viral genome the inserted mini-genes are expressed from the strong vaccinia p<sub>7.5</sub> promoter.

Intracellular trafficking signals added to peptides.

Short amino acid sequences can act as signals to target proteins to specific intracellular compartments. For example, hydrophobic signal peptides are found at the 30 amino terminus of proteins destined for the ER, while the sequence KFERQ (and other closely related sequences) is known to target intracellular polypeptides to lysosomes, while other sequences target polypeptides to endosomes. In addition, the peptide sequence KDEL has been shown to act as a retention signal for the ER. Each of these

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signal peptides, or a combination thereof, can be used to traffic the immunomodulating peptides of the invention as desired. For example, a construct encoding a given immunomodulating peptide linked to an ER-targeting signal 5 peptide would direct the peptide to the ER, where it would bind to the class II molecule as it is assembled, preventing the binding of intact Ii which is essential for trafficking. Alternatively, a construct can be made in which an ER retention signal on the peptide would help 10 prevent the class II molecule from ever leaving the ER. If instead a peptide of the invention is targeted to the endosomic compartment, this would ensure that large quantities of the peptide are present when invariant chain is replaced by processed peptides, thereby 15 increasing the likelihood that the peptide incorporated into the class II complex is the high-affinity peptides of the invention rather than naturally-occurring, potentially immunogenic peptides. The likelihood of peptides of the invention being available incorporation 20 into class II can be increased by linking the peptides to an intact Ii polypeptide sequence. Since Ii is known to traffic class II molecules to the endosomes, the hybrid Ii would carry one or more copies of the peptide of the invention along with the class II molecule; once in the 25 endosome, the hybrid Ii would be degraded by normal endosomal processes to yield both multiple copies of the peptide of the invention or molecules similar to it, and an open class II binding cleft. DNAs encoding immunomodulatory peptides containing targeting signals 30 will be generated by PCR or other standard genetic engineering or synthetic techniques, and the ability of these peptides to associate with DR molecules will be analyzed in vitro and in vivo, as described below.

It is proposed that the invariant chain prevents 35 class II molecules from binding peptides in the ER and

may contribute to heterodimer formation. Any mechanism that prevents this association would increase the effectiveness of class II blockade. Therefore, a peptide corresponding to the site on Ii which binds to the class II heterodimer, or corresponding to the site on either the  $\alpha$  or  $\beta$  subunit of the heterodimer which binds to Ii, could be used to prevent this association and thereby disrupt MHC class II function.

In Vitro Assembly.

Cell free extracts are used routinely for 10 expressing eukaryotic proteins (Krieg, P. & Melton, D. (1984) Nucl. Acids Res. 12, 7057; Pelham, H. and Jackson, R. (1976) Eur. J. Biochem. <u>67</u>, 247). Specific mRNAs are transcribed from DNA vectors containing viral RNA 15 polymerase promoters (Melton, D. et al. (1984) Nucl. Acids Res. 12, 7035), and added to micrococcal nucleasetreated cell extracts. The addition of 35S methionine and amino acids initiates translation of the exogenous mRNA, resulting in labeled protein. Proteins may be 20 subsequently analyzed by SDS-PAGE and detected by autoradiography. Processing events such as signal peptide cleavage and core glycosylation are initiated by the addition of microsomal vesicles during translation (Walter, P. and Blobel, G. (1983), Meth. Enzymol., 96, 25 50), and these events are monitored by the altered mobility of the proteins in SDS-PAGE gels.

The ability of peptides containing a signal peptide sequence to be accurately processed and to compete with invariant chain for class II binding in the 30 ER are assayed in the *in vitro* system described above. Specifically, DR1 $\alpha$  and  $\beta$  chain, and invariant chain peptide constructs described above are transcribed into mRNAs, which will be translated in the presence of mammalian microsomal membranes. Association of the DR

heterodimer with Ii is determined by immunoprecipitation with antisera to DR and Ii. Addition of mRNA encoding the peptide of the invention to the translation reaction should result in a decreased level of

5 coimmunoprecipitated Ii, and the concomitant appearance of coimmunoprecipitated peptide, as determined by SDS-PAGE on TRIS-Tricine gels. These experiments will provide us with a rapid assay system for determining the potential usefulness of a given blocking peptide as a competitor for Ii chain binding in the ER. Those peptides of the invention which prove to be capable of competing successfully with Ii in this cell-free assay can then be tested in intact cells, as described below.

In Vivo Assembly.

Human EBV-transformed B cell lines LG-2 and HOM-2 (homozygous for HLA-DR1) and the mouse B cell hybridoma LK35.2 are transfected with either 50μg of linearized pHβactin-α-Ii<sub>15</sub> or pHβactin-α-Ii<sub>24</sub> or (as a control) pHβactin-1-neo by electroporation (150mV, 960μF, 0.2cm cuvette gap). Following electroporation, the cells are cultured in G418-free medium until total recovery (approximately 4 days). Each population is then placed under G418 selection until neomycin-expressing resistant populations of transfectants are obtained (approximately 1-2 months). The resistant populations are subcloned by limiting dilution and the clonality of stable transfectants determined by PCR amplification of blocking peptide mRNA expression.

Stable transfectants of LG-2 and HOM-2 carrying
30 blocking peptide mini-genes or negative control vectors
are grown in large-scale culture conditions until 20
grams of pelleted cell mass is obtained. The HLA-DR
expressed by each transfectant is purified, and the bound
peptide repertoire (both from within the cell and from

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the cell surface) analyzed as described above. Successful demonstration of a reduction in the total bound peptide diversity will be conclusive evidence of intracellular delivery of immuno-modulatory peptides.

A second cell-based assay utilizes stable 5 transfectants of LK35.2 cells carrying blocking peptide mini-genes or negative control vectors; these cells are used as APCs in T cell proliferation assays. Each transfectant is cultured for 24 hours in the presence of 10 different dilutions of hen egg lysozyme (HEL) and HELspecific T cell hybridomas. The relative activation of the T cells present in each assay (as measured by lymphokine production) is determined using the publicly available lymphokine dependent cell line CTLL2 in a 3H-15 thymidine incorporation assay (Vignali et al. (1992) J.E.M. 175:925-932). Successful demonstration of a reduction in the ability of blocking peptide expressing transfectants to present HEL to specific T cell hybridomas will be conclusive evidence of intracellular 20 delivery of immuno-modulatory peptides. Cells of the human TK cell line 143 (ATCC) are infected with vaccinia virus (strain WR, TK+) (ATCC), and two hours postinfection, pSC11- $\alpha$ -Ii<sub>15</sub> or pSC11- $\alpha$ -Ii<sub>24</sub> or pSC11 is introduced into the infected cells by calcium phosphate 25 precipitation. TK recombinants are selected for with bromodeoxyuridine at  $25\mu g/ml$ . Recombinant plaques are screened by PCR for the presence of mini-gene DNA. Recombinant virus is cloned by three rounds of limiting dilution to generate pure clonal viral stocks.

In experiments analogous to the transfection experiments described above, recombinant vaccinia viruses encoding mini-genes or vector alone will be used to infect large-scale cultures of the human EBV transformed B cell lines LG-2 and HOM-2. Following infection, the 35 HLA-DR is purified and the bound peptide repertoire

30

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analyzed as described above. A reduction of the complexity of the bound peptide population and a significant increase in the relative amount of Ii peptides bound are conclusive evidence that vaccinia can deliver blocking peptides to human APCs.

The same recombinant vaccinia viruses encoding mini-genes or vector will be used to infect mice experiencing experimentally-induced autoimmunity. A number of such models are known and are referred in 10 Kronenberg, Cell 65:537-542 (1991).

Liposomal Delivery of Synthetic Peptides or Mini-gene Constructs.

Liposomes have been successfully used as drug carriers and more recently in safe and potent adjuvant strategies for malaria vaccination in humans (Fries et al. (1992), Proc. Natl. Acad. Sci. USA 89:358).

Encapsulated liposomes have been shown to incorporate soluble proteins and deliver these antigens to cells for both in vitro and in vivo CD8+ mediated CTL response (Reddy et al., J. Immunol. 148:1585-1589, 1992; and Collins et al., J. Immunol. 148:3336-3341, 1992). Thus, liposomes may be used as a vehicle for delivering synthetic peptides into APCs.

Harding et al. (Cell (1991) 64, 393-401) have

25 demonstrated that the targeting of liposome-delivered antigen to either of two intracellular class II-loading compartments, early endosomes and/or lysosomes, can be accomplished by varying the membrane composition of the liposome: acid-sensitive liposomes were found to target their contents to early endosomes, while acid-resistant liposomes were found to deliver their contents to lysosomes. Thus, the peptides of the invention will be incorporated into acid-sensitive liposomes where delivery

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to endosomes is desired, and into acid-resistant liposomes for delivery to lysosomes.

Liposomes are prepared by standard detergent dialysis or dehydration-rehydration methods. 5 sensitive liposomes, dioleoylphosphatidylethanolamine (DOPE) and palmitoylhomocystein (PHC) are utilized, while dioleoylphospatidylcholine (DOPC) and dioleoylphosphatidylserine (DOPS) are used for the preparation of acid-resistant liposomes. 10<sup>-5</sup> mol of total 10 lipid (DOPC/DOPS or DOPE/PHC at 4:1 mol ratios) are dried, hydrated in 0.2 ml of HEPES buffered saline (HBS) (150 mM NaCl, 1 mM EGTA, 10mM HEPES pH 7.4) and sonicated. The lipid suspensions are solubilized by the addition of 0.1 ml of 1 M octylglucoside in HBS. 15 peptides to be entrapped are added to 0.2 ml of 0.6 mM peptide in 20% HBS. The mixture is then frozen, lyophilized overnight, and rehydrated. These liposomes will be treated with chymotrypsin to digest any surfacebound peptide. Liposome delivery to EBV-transformed cell 20 lines (as described above) will be accomplished by 12-16 hour incubation at 37°C. HLA-DR will be purified from the liposome treated cells and bound peptide analyzed as above.

Alternatively, the liposomes are formulated with 25 the DNA mini-gene constructs of the invention, and used to deliver the constructs into APCs either <u>in vitro</u> or <u>in vivo</u>.

Human immunization will be carried out under the protocol approved by both The Johns Hopkins University

30 Joint Committee for Clinical Investigation and the Human Subject Research Review Board of the Office of the Surgeon General of the U.S. Army (Fries et al. (1992), P.N.A.S. U.S.P. 89:358-362), using dosages described therein, or other dosages described in the literature for liposome-based delivery of therapeutic agents.

Delivery via Immune-stimulating Complexes (ISCOMS).

ISCOMS are negatively charged cage-like structures of 30-40nm in size formed spontaneously on mixing cholesterol and Quil A (saponin). Protective immunity 5 has been generated in a variety of experimental models of infection, including toxoplasmosis and Epstein-Barr virus-induced tumors, using ISCOMS as the delivery vehicle for antigens (Mowat and Donachie) Immunology Today 12:383-385, 1991. Doses of antigen as low as  $1\mu g$ 10 encapsulated in ISCOMS have been found to produce class I mediated CTL responses, where either purified intact HIV-1-IIIB qp 160 envelope glycoprotein or influenza hemagglutinin is the antigen (Takahashi et al. , Nature 344:873-875, 1990). Peptides are delivered into tissue 15 culture cells using ISCOMS in a manner and dosage similar to that described above for liposomes; the class II peptide binding of delivered peptides are then determined by extraction and characterization as described above. ISCOM-delivered peptides of the invention which are 20 effectively utilized by cultured cells are then tested in animals or humans.

In addition to delivery of the therapeutic synthetic peptides, ISCOMS could be constituted to deliver the mini-gene constructs to APCs, and thus serve as an alternative to the above-outlined vaccinia strategy.

Immunogenic Peptide Delivery (Vaccines).

In addition to using the above-described intracellular delivery systems to deliver nonimmunogenic self peptides with the specific aim of down-modulating the immune system (thus alleviating autoimmune conditions), the delivery systems of the invention may alternatively be used as a novel means of vaccination, in order to <a href="stimulate">stimulate</a> a portion of the immune system of an

animal. In the latter context, the delivery system is employed to deliver, into appropriate cells, DNA constructs which express immunogenic, pathogen-derived peptides intended to stimulate an immune response against 5 a specific pathogen. Because the antigenic peptide is produced inside the target cell itself, the vaccine method of the invention ensures that there is no circulating free antigen available to stimulate antibody formation and thereby induce potentially deleterious or 10 inappropriate immunological reactions. The immune response stimulated by vaccines of the invention is, because the vaccines are targeted solely to APC's, limited to the T cell mediated response, in contrast to standard vaccine protocols which result in a more 15 generalized immune response. Although some of the peptide-presenting APC's will initially be lysed by host T cells, such lysis will be limited because, inter alia, the virus-based vaccine is non-replicative, i.e., each carrier virus can infect only one cell.

The model antigen that will be used to perfect and 20 test the system of the invention is hen egg lysozyme (HEL). It is arguably the most well characterized protein for antigen presentation studies, to which there are numerous monoclonal antibodies and class I- and class 25 II-restricted mouse T cell clones and hybridomas. primary epitopes that will be studied are the peptide HEL 34-45, as both monoclonal antibodies and CD4+ T cell hybridomas are available, and peptide HEL 46-61, as both class I and class II-restricted T cell clones and 30 hybridomas have been raised and are publicly available. These two sequences are thus proven immunogenic epitopes. Initially, four constructs encoding different polypeptides are analyzed: (a) whole, secreted HEL, (B) HEL 34-45, (c) HEL 46-61, and (d) HEL 34-61. The last 35 three include a signal sequence known to be cleaved in

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these cells, e.g., IAk (MPRSRALILGVLALTTMLSLCGG), which would result in targeting to the ER. All constructs are then subcloned into pH\$Apr-1 neo. The methodology for making these constructs is similar to that outlined The constructs are introduced into appropriate APCs, e.g., LK35.2 cells, by means of a conventional eukaryotic transfection or one of the delivery vehicles discussed above (e.g., vaccinia, liposomes, or ISCOMS). LK35.2 cells, which possess the mouse MHC Class II 10 restriction molecules IAk and IEk, transfected with each of the constructs are tested for their ability to stimulate the appropriate class I and class II-restricted T cell hybridomas and clones using standard techniques. Whether class I stimulation is observed will depend on 15 whether peptide trimming can occur in the ER, in order to produce an 8-10-mer suitable for binding to class I molecules. If these constructs are ineffective for class I stimulation, they can be modified in order to produce a more effective peptide for class I binding. If these 20 constructs prove to be less effective for class IIrestricted responses, they can be tagged with endosomal and/or lysosomal targeting sequences as discussed in Section V.

The effectiveness of targeting signals used to
25 direct immunogenic peptides to particular intracellular
organelles would be monitored using electron microscopic
analysis of immunogold stained sections of the various
transfectants. Rabbit anti-peptide antisera would be
produced and affinity purified for this application. In
30 addition, monoclonal antibody HF10, which recognizes HEL
34-45, will be used.

Once a construct is defined that can be effectively presented by transfectants in vitro, its effectiveness in vivo will be determined. This can be tested by injection of the transfectants i.p. and/or s.c.

into C3H/Balb/c Fl mice, or by injection of the construct incorporated into an appropriate delivery vehicle (e.g., liposome, ISCOMS, retrovirus, vaccinia). Optimal protocols and doses for such immunizing injections can be determined by one of ordinary skill in the art, given the disclosures provided herein. Efficiency of immunization can be tested by standard methods such as (a) proliferation of class II-restricted T cells in response to HEL pulsed APCs, (b) CTL response to <sup>51</sup>Cr-labeled targets, and (c) serum antibody titre as determined by ELISA.

Once the details of the vaccine delivery system of the invention are optimized, constructs encoding peptides with useful immunizing potential can be incorporated into 15 the system. Such peptides can be identified by standard means now used to identify immunogenic epitopes on pathogen-derived proteins. For example, candidate peptides for immunization may be determined from antibody and T cell analysis of animals infected with a particular 20 pathogen. In order to obtain a protective and effective anamnestic response, the peptides used for vaccination should ideally be those which are presented with the highest frequency and efficiency upon infection. This could best be determined by using the procedures outlined 25 in the experimental section above to extract and characterize the peptides bound by MHC class II molecules from infected cells. Given allelic restriction of immunogenic peptides (in contrast to the observed degenerate binding of self peptides of invention), a 30 mini-gene encoding several immunogenic peptides will probably be required to provide a vaccine useful for the entire population. Vaccine administration and dosage are as currently employed to smallpox vaccination.

What is claimed is:

TABLE 1 LG-2/HLA-DR1 BINDING PEPTIDES

HLA-A2 11							
ant Chain	101-120	VGSDUBEI BGYHOYAYDG	16	DR15-59	2190.4	2190.4	39.5
1 1 1	103-120	ACCUPATION OF THE PROPERTY OF	- 51	DR15-58	1855.0	1854.4	5.706
1 1	711-E01	ACRACIO DI CONTROLLA	- 71	DR15-58	1784.0	1783.6	53.3
1 1	103-110	ANGUAL ENGLANDES	. 7	DR15-56	175.3	1755.2	8.5
rient Chain	104-117 105-117	SDURFLEGYHOYA	: <b>£</b> 1	DR15-56	1698.2	1698.8	2.82
rient Chain		SMG I FOR 1 101 ENGRASSION OF	52	DR15-88	2733.5	2734.5	40.5
	071-06	· OKDOKOVEKNOMATDI (MOA) DH	54	DR15-88	2676.4	2675.9	80.8
	yo-119	OKDOKOVSKUDNATO I MOA! PUG	52	DR15-86	2620.2	2619.7	91.5
	071-76	O PROPERTY CANDING TO SELECT THE	23	DR15-86	2545.2	2544.5	112.2
	94-110	OVODNOVEKNOMATO! I MOA! DM	: X	DR15-87	2563.2	2562.3	145.0
	411-74	MO TANDES OF THE PARTY OF THE P	52	DR15-87	2466.1	2465.8	101.5
	98-119	DYDOXOUSEMBHATD!	22	DR15-84	2432.0	2431.7	72.5
	911-76	O TACM I INTERNATIONAL OF	77	DR15-84	2334.9	2334.2	31.6
	811-86	O TO COMPANY TO THE OWNER OF THE OWNER OWN		DR15-86	2206-7	2207.4	89.8
	98-118	PPRFVSKARANIFILITANIF	5 2	DR15-88	1732.2	1731.9	178.5
. •	105-119 105-118	KMRMATPLLMOALP	71	DR15-86	1601.0	1600.2	162.0
Na+/K+ AlPase	199-216	I PADLR I I SANGCK VDNS	81	DR15-56	1886.6	1885.8	48.8
Transferrin Recpt.	969-089	RVEYHFLSPYVSPKESP	71	DR15-58	2035.3	2036.8	30.3
	i	100000000000000000000000000000000000000	õ	DR15-51	2237.6	2236.5	0.89
Bovine Fetuin	56-73 56-73	YKHTLNGIDSVKVWPRRP	82	DR15-50	2338.7	2338.5	32.5
HLA-DR β-chain	19-61	DVGEYRAVTELGRPDAEYW	91	DR1S-51	2226.5	~	
Carboxypeptidase E	101-115	EPGEPEFKY I GNMHG .	<b>21</b>	DR15-48	1704.9	1700.4* ESI-MS	

# SUBSTITUTE SHEET

TABLE 2
PEPTIDE BINDING TO HLA-DR1

PEP110E <sup>8</sup>	LENGTH	Ki vs HA 307-319 <sup>b</sup>	SDS-Resistance <sup>C</sup> rM
		#1	
HLA-A2 103-117	15	£ ∓ 67	•
11 105-119	15	< 10	•
11 96-119	54	33 ± 5	•
N8+/K+ ATPBSe 199-216	18	68 ± 9	•
Transf. Recept. 680-696	11	< 10	•
Bovine Fetuin 56-72	19	66 ± 18	•
HA 307-319	71	< 10	•
11 96-110	51	, 104	
β <sub>2</sub> m 52-64	13	, 10 <sup>4</sup>	ı

The first six entries correspond to peptides found associated with HLA-DR1 and the sequences are shown in Table 1. Two control peptides were U invariant chain derived peptide isolated from HLA-DR1. Peptides were synthesized using solid-phase Fmoc chemistry, deprotected and cleaved also tested:  $eta_2$ m 52-64, SDLSFSKDWSFYL, is from human  $eta_2$ -microglobulin and Ii 96-110, LPKPPKPVSKMRMAI is a truncated version of the longest using standrd methods, then purified by RPC. Purified peptides were analzyed by mass spectrometry and concentrations were determined by quantitative ninhydrin analysis.

Specific activity, determined by BCA assay (Pierce) and gamma counting, was 26,000 cpm/pmol. 10nM labeled peptide and 10 nM purified HLA-DR1 four hour exposures on the phospho-imaging plates. Percent inhibition was calculated as the ratio of background-corrected radioactivity in Inhibition constants (Ki) were measured as the concentration of test peptide which inhibited 50% of the <sup>125</sup>I-labeled HA 307-319 binding to separated by native gel electrophoresis (33) and bound radioactivity was quantitated using a Fujix imaging plate analyzer (BAS 2000) after "empty" HLA-DR1 produced in Sf9 insect cells (20). MA 307-319 was labeled using Na<sup>125</sup>1 and chloramine-T and isolated by gel filtration. were mixed with 10 different concentrations (10 nM to 10 MM) of synthetic cold competitor peptide in phosphate-buffered saline, pM 7.2, containing 1 mM EDIA, 1mM PMSF, 0.1 mM iodoacetamide, and 3 mM NaN3, and incubated at 37°C for 85 hours. Free and bound peptide were the sample to background-corrected radioactivity in a parallel sample containing no competitor peptide. Under these conditions, Ki measurements < 10 nM could not be accurately determined.

PAGE with and without boiling prior to loading. Peptides which prevented SDS-induced chain dissociation are indicated positive (+) and those phosphate-buffered saline (pH 7.2) with the protease inhibitor mixture described above. After incubation, the samples were analyzed by SDSdetermined as described (20). Briefly, 20 μM HLA-DR1 was incubated with five-fold excess of synthetic peptide at 37°C for 85 hours, in the ability of the synthetic peptides to confer resistance to SDS-induced chain dissociation of HLA-DR1 produced in insect cells was that did not negative (-).

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TABLE 3 - PUTATIVE HLA-DR1 PEPTIDE BINDING NOTIF

HILL + A.   SOUGELBECTHAIN   13   105-117   This stody	A PROTEIN SOURCE	PEPTIDE SEQUENCE	LENGTH	POSITION	REFERENCE	
14   105-118   14   105-118   14   105-118   14   105-118   14   105-118   14   105-116   14   105-116   14   105-116   14   14   14   14   14   14   14	HLA-A2	SDURFLRGYHOYA	13	105-117	This study	
199-216   1900LR ILSANGCKYONS   18   199-216	Invariant Chain	KMRMAIPLLMOALP	7,	105-118		
ine Fetuin         RECEPTOR         REGOUNDS WINNERS         17         680-696           ine Fetuin         YKHILNQIOS WINNERS         18         56-73           ine Fetuin         KYKATLINQIOS WINNERS         18         1-18           ine Fetuin         KYKATLINGIOS WINNERS         16         112-129           ine RHILGINGIANGIA         16         112-129           ine RHILGINGIANGIA         16         19-34           ine REGREKHIDAGCRTH         16         19-34           ine ASTIKLIKEPALLIKATOG         20         11-30           p17         CARRISTIKLIKEPALLIKAT         20         11-30           p18         ASTIKLIKEPALLIKAT         20         11-10           p17         CARRISTIKLIKE         16         97-112           p17         CARRISTICALIVO         20         111-130           p18         RILVORNIGITIKAT         15         249-263           tactop. p190         LKKUFGERFLIKAT         15         249-263           tactop. p190         LKKUFGERFLIKAT         15         307-319           tactop. p190         LKKUFGERFLIKAT         15         240-263           tactop. p190         LKKUFGERFLIKAT         15         26-263	Na+/K+ ATPase	I PADLR I SANGCKYDNS	18	199-216		
1-16   ΓΕΓΙΙΙΙ   112-129   1-18	Transferrin Receptor		17	969-089		
gveggeladamkrhold         18         1-18           Raugckstdvalerach         18         112-129           HPHIEJOHLKHGKKI         16         31-46           HPHIEJOHLKHGKKI         16         19-34           SKRVYGMFDLRKY         14         115-128           ATSTKLHKEPATLIKAIDG         20         1-20           p1         DRVKLHYEGAPLIKAKGQ         20         11-30           p13         ORVKLHYEGAPLIKAKGQ         20         111-130           p17         GARASULEGALIKAKGQ         20         11-130           p17         GARASULEGALIKAK         20         11-130           p17         GARASULEGALIKAK         16         97-112           p18         RILYONYONITKAK         13         307-319           telerip, D190         LKKLVFGTRKPLDNI         15         249-263           feletip, CS         KATKOTRKPLNI         13         329-341           feletip, CS         KATROZILKKAK         15         249-263           feletip, CS         KATROZILKKIKAK         15         369-341           g chain         GOTREPREJEKELFRU         20         1-20           g chain         GOTREPREJEKELFRU         20         1-20	Bovine Fetuin		18	56-73		
RABCKGTDVANAIRGCRL   16   112-129	HE.	KVFGRCELAAAMKRHGLD	18	1-18	9	
HPPHIEIOMLKNOKKII   16   31-46   16   18-34   16   18-34   16   18-34   16   18-34   16   18-34   16   18-34   16   18-34	<u> </u>	RNECKGTDVQAUIRGCRL	18	112-129	•	
NELGREKHTDA_CCRTH	E	HPPHIEIGMLKNGKKI	16	31-46	9	
SKENCYQHFDLRKY         14         115-128           ATSTKLHKEPATLIKAIDG         20         1-20           PATLIKALDGDTVKLHYKGG         20         11-30           DRVKLMYGGAPHIFRILLVD         20         21-40           VAYVYKPHNTHEGHLRSEA         20         111-130           QKGEPIDKELYPLTSL         16         97-112           CARASVLEGGELDKNE         16         17-12           CARASVLEGGELDKNE         16         187-206           PKTVKQHITKLAT         13         307-319           LKKLVFGYRKPLDNI         15         249-263           KHIEQYLKKIKNS         13         329-341           DVFKELKYHANABHIE         16         15-30           GDT-REVELKYHANABHIE         20         1-20           TERVRILEBCITYUGEESYFFIG         20         1-20           TERVRILEBCITYUGEESYFFIG         20         1-20           KARRADLIAYLKGATAK         17         88-104           KARRADLIAYLKGATAK         17         88-104           KARRADLOGGAPHIT         16         15-31           PULKARIADREHANITER         16         17-20           ROLLGUOPELPONTER         17         88-104           ROLLGUOPELPONTER         16         17-20	μ2''' PI <b>A</b> -	NELGREKHTDACCRTH	16	19-34	9	
ATSTKKLHKEPATLIKAIDG         20         1-20           PATLIKAIDGQIVKLMYKGQ         20         11-30           DRVKLMYKGQPMIFRILLUD         20         21-40           VATVYKPUNTIHEQHLEKSEA         20         111-130           QKQEPIDKELYPLTSL         16         97-112           GARASVLŞGGELDKME         16         97-112           GARASVLŞGGELDKME         20         187-206           PKYVKQHTKLAT         13         307-319           LKKLVFGYRKPLDNI         15         249-263           KHIEQYLKKINS         15         249-263           LKKLVFGYRKINS         15         329-341           DVKELLKHMANENIE         16         15-30           GDVKELLKHNG         20         1-20           TERVRILLERCIYNGESEYENG         20         1-20           TERVRILLERCIYNGESEYENG         20         1-20           KARRAQLIAYLKQATAK         17         88-104           KARRAQLIAYLKWINYPRTPPP         24         75-98           PRIKAEINQUESE         16         7-22           IQVYSRHPPELGEPVIGGS         16         89-104           RQILGQLOPŞLOTGSE         16         7-22           INTYSRHPPELGHPUGGG         16         89-100 <td>2</td> <td>SKPKVYQUFDLRKY</td> <td>71</td> <td>115-128</td> <td>9</td> <td></td>	2	SKPKVYQUFDLRKY	71	115-128	9	
PATLIKAIOGQIVKLMYKGQ         20         11-30           DRVKLMYKGOPMIFRILLVD         20         21-40           VATVYKPMNTHEQHLEKSEA         20         111-130           QKQEPIDKELTPLTSL         16         97-112           GARASVLSGGELDKNE         16         97-112           GARASVLSGGELDKNE         16         1-16           RILYOMVGTYUSVGTSTLMK         20         187-206           PKYVKOMITKLAAT         13         307-319           LKKLVFGTKKPLANI         15         249-263           KHIEQYLKKLKNS         13         329-341           DVFKELKVHAMENIE         16         15-30           GOTEPRELKCHFRIG         20         1-20           TERVALLERCIYNQEESYRFD         22         21-42           DLLEGOREMAVIEKNIVTPRTPPP         24         75-98           PLKAERAQLIATLKATAK         17         88-104           KAERAQLIATLKATAK         17         88-104           ROLEGOREMOVER         16         57-72           ROLLGOLOGLOGE         16         57-72           IQVYSEHPPENGKRMI         16         88-100           ROLLGOREMORPIS         16         7-22           ROLLGOREMORPHI         16         85-100 </td <td>NASE</td> <td>ATSTKKLHKEPATLIKAIDG</td> <td>20</td> <td>1-20</td> <td>9</td> <td></td>	NASE	ATSTKKLHKEPATLIKAIDG	20	1-20	9	
DRVKLHYKGAPMIFRILLUD         20         21-40           VATVYKPHNITHEGHLRKSEA         20         111-130           GARASVLSGGELDKWE         16         97-112           GARASVLSGGELDKWE         16         1-16           RILYQHVGTYVSVGISTLMK         20         187-206           PKYVKQHILKLAI         13         307-319           LKKLVFGYRKPLDHI         15         249-263           LKKLVFGYRKPLDHI         15         249-263           LKKLVFGYRKPLDHI         15         249-263           DVFKELKYHHAMENIE         16         15-30           GDIRFRLLMALEECTHFUG         20         1-20           TERVRLLERCTYNGESYRDS         22         21-42           DLLEGRRANDITCRHHYGGEST         25         21-42           LEGRRADLIAILKKAITKGATAK         17         88-104           KAERAQLIAILKGATAK         17         88-104           KAERAQLIAILKGUTAK         13         19-31           PLKAEIAQUEEDV         13         19-31           RQILGQLOPSLATGE         16         57-72           RQILGQLOPSLAPLIGGG         16         85-100		PATL IKAIDGDTVKLMYKGQ	80	11-30	9	
VATVYKPHNTHEGHLÆKSEA         20         111-130           QKGEPIDKELYPLTSL         16         97-112           GARASVLÆGGELDKNE         16         1-16           RILYGHVGTYSVGTSTLHK         20         187-206           PKYVKGHTKLAT         13         307-319           LKKLVFGTRREDNI         15         249-263           LKKLVFGTRREDNI         15         249-263           GDTKELKYHHAMENIE         16         15-30           GDTRPRLLAGLÆECHFING         20         1-20           TERVRLLEECTYNQEESFT         22         21-42           DLEGARBANDITKRANTKRATAK         17         88-104           KAERAQLIAYLKGATAK         17         88-104           L.         GRIGOGENPVHFFKNIVTPRTPPP         24         75-98           PLKAEIAQRLEQV         13         19-31           PLKAEIAQRLEQV         16         57-72           ROLYSRHPPENGKPHI         16         85-100           INTECYKLEHPVLGGG         16         85-100		DRVKLMYKGOPMIFRLLLVD	50	21-40	9	-
QKQGEPIQKELYPLTSL         16         97-112           CARRASVLSGGELDKAE         16         1-16           RILYQQUGTYVSVGTSTLHK         20         187-206           PKYVKQHILKLAT         13         307-319           LKKLVFGYRKPLDNI         15         249-263           KHIEQYLKKINS         13         329-341           DVFKELKYHHAMENIE         16         15-30           GDTRPRLLAGLKECHFNG         20         1-20           TERVRLLEBCTYNQEESYRFDS         22         21-42           DLLEGRRAVDIYCRHYRVGGESFT         25         66-90           KARRAQLIAYLKGATAK         17         88-104           KARRAQLIAYLKGATAK         17         88-104           RAGRAQLIAYLKGATAK         17         88-104           RAGRAQLIAYLKGATAK         17         88-104           RAGRAQLIAYLKGATAK         13         19-31           PLKAEIAQRLEDV         13         19-31           RQIOOSEMPOWHERWINTPRINGKPUI         16         57-72           RQIOVYSRHPPENGKPUI         16         85-100           INTECYKLEHPUIGGG         16         85-100		VAYVYKPHNTHEGHLRKSEA	20	111-130	•	-36
GARASULŞGGELDKAE         16         1-16           RILYQNUGIYUSUGISTLAK         20         187-206           PKYVKQHILKLAT         13         307-319           LKKLVFGYRKPLDNI         15         249-263           LKKLVFGYRKPLDNI         15         249-263           KHIEQYLKKIKNS         13         329-341           DVFKELKVHAMENIE         16         15-30           TERVALLERCIFNGESFIFIG         20         1-20           TERVALLERCIYNGESFIFIG         20         1-20           JAFRAQLIAYLKATAK         17         88-104           KAFRAQLIAYLKATAK         17         88-104           L. GRTODENPVHFFKNIVTPRTPPP         24         75-98           PLKAEIAQRLEQU         13         19-31           PLKAEIAQRLEQU         16         57-72           RQILGQLOPŞLQIGSE         16         7-22           IOVYSRHPPENGKPMI         16         85-100		QKOEPIDKELYPLTSL	16	97-112	•	<del>-</del>
RILYON/GISTLNK         20         187-206           PKYVKON/TUKLAT         13         307-319           LKKLVFGYRKPLDNI         15         249-263           KHIEGYLKKIKNS         13         329-341           DVFKELKVHANMENIE         16         15-30           GOTRPRFLYALKEKINF         20         1-20           TERVRLLERCIYNQEESFT         22         21-42           DLLEGARBANDIYCRHNYGVGESFT         25         66-90           KAERAQLIAYLKOATAK         17         88-104           L.         GRTODENPVHFFKNIVTPRTPPP         24         75-98           PLKAEIAQRLEQV         13         19-31           PLKAEIAQRLEQV         16         57-72           ROLIGOLOPSLATGGS         16         85-100           INTKCYKLEHPVIGGG         16         85-100	HIV D17	GARASVLSGGELDKWE	16	1-16	9	
PKTVKQHTLKLAT	Influenza HA	RILYQHVGTYVSVGTSTLHK	50	187-206	9	
LKKLVFGYRKPLDNI         15         249-263           KHIEGYLKKIKNS         13         329-341           DVFKELKYHRAMENIE         16         15-30           GDTRPRLYGHKECHFNG         20         1-20           TERVALLERCIYNGESYRFDS         22         21-42           DLLEGRRAADIYCRHINFGVGESFT         25         66-90           KAERAQLIAIKKAATAK         17         88-104           L. GRIGOEMPVHFFKNIVTPRTPPP         24         75-98           PLKAEIAQRLEDV         13         19-31           ROLLGOLOPSLOTGSE         16         57-72           ROLLGOLOPSLOTGSE         16         85-100           INTKCYKLEHPVIGGG         16         85-100	Influenza HA	PKYVKQHTLKLAT	13	307-319	21.	
KAHIEQYLKKIKNS         13         329-341           DVFKELKVHHAMENIE         16         15-30           GOTRPRFLYQUKECHFNG         20         1-20           TERVALLERCIYNQEESFT         22         21-42           DLLEGREANDIYCRHWGWGESFT         25         66-90           KAERAQLIAYLKGATAK         17         88-104           L. GRTGDENPVHFFKNIVTPRTPPP         24         75-98           PLKAEIAQRLEQV         13         19-31           PLKAEIAQRLEQV         16         57-72           ROLLGQLOPELGGE         16         85-100           INTKCYKLEHPVIGGG         16         85-100	P. falcip, p190	LKKLVFGYRKPLDNI	15	249-263	23	
DVFKELKYNHAMENIE         16         15-30           GDTRPRLLAGLKECHFNG         20         1-20           TERVALLEECTYNQEESYRPS         22         21-42           DLLEGARRAVDIYCRHWYGVGESFT         25         66-90           KAERAQLIAYLKQATAK         17         88-104           t. GRIODEMPVHFKNIVTPRTPP         24         75-98           PLKARIAGNLEQV         13         19-31           ROLLGQLOPELGIGSE         16         57-72           IOVYSRHPPENGKPNI         16         7-22           INTECYKLEHPVIGGG         16         85-100	P. falcip. CS	KHIEQYLKKIKNS	51	329-341	22	
GDTRPRFLYQLKEECHFRIG         20         1-20           TERVALLERCIYNQEESYRFDS         22         21-42           DLLEGRRAAVDIYCRHINVGGESFT         25         66-90           KAERAQLIAILKGATAK         17         88-104           L. GRIGOENPVYHFKNIVTPRTPPP         24         75-98           PLKAEIAQRLEQV         13         19-31           RQILGQLOPŞLQIGSE         16         57-72           IQVYSRHPPENGKPNI         16         7-22           INTKCYKLEHPVIGGG         16         85-100	Chicken OVA	DVFKELKVHHANENIE	16	15-30	•	
TERVRLLERCIYNQEESUREDS         22         21-42           DLLEGRRANDIYCRHWYGVGESFT         25         66-90           KAERAQLIAYLKOATAK         17         88-104           t. GRICOEMPVHFKMIVTPRTPPP         24         75-98           PLKAEIAQRLEQV         13         19-31           ROLLGQLOPSLOTGSE         16         57-72           IOVYSRHPPENGKPNI         16         7-22           INTKCYKLEHPVIGGG         16         85-100	081 A chain	GOTRPRFLWOLKFECHFFNG	20	1-20	28	
DILLEGREAAVDITCRHUNGUGESFT         25         66-90           KAERAQLIAYLKGATAK         17         88-104           L. GRTODEMPUVHFKNIVTPRTPPP         24         75-98           PLKAEIAQRLEQV         13         19-31           ROILGQLOPELOTGE         16         57-72           IOVYSRHPPEMCKPMI         16         7-22           INTKCYKLEHPVIGGG         16         85-100		TERVELLERCIYNOEESURFDS	22	21-42	28	
EAGRAQLIAILKQATAK  1. GRIODENPVVHFFKNIVTPRTPPP 24 PLKAEIAQRLEQV RQILGQLQPELQTGSE 10 VYSRHPPENGKPNI 11 INTKCYKLEHPVIGGG		DLLEGRRAAVDIYCRHWYGVGESFT	23	06-99	28	
t. GRIGOEMPVVHFFKNIVIPRIPPP 24 PLKAEIAQRLEQV 13 RQILGQLOPSLQIGSE 16 IQVYSRHPPEHGKPMI 16 INTKCYKLEHPVIGGG	b Cvt c	KAERADLIAYLKQATAK	17	88-104	9	
PLKAETAORLEDV 13 ROTLGOLOPSLATGSE 16 TOVYSRHPPEHGKPMI 16 INTKCYKLEHPVIGGG 16	Myelin basic prot.	GRIODEMPVVHFFKNIVIPRIPPP	54	75-98	9	
ROILGOLOPELOTGSE 16 IOVYSRHPPENGKPMI 16 INTECYKLEHPVIGGG 16	Influenza Matrix	PLKAETAORLEDV	13	19-31	9	
I OVYSRHPPENGKPNI I NYKCYKLEHPVIGG 16	HIV DIT	ROILGOLOPSLOTGSE	16	21-72	9	
INTKCYKLEHPVIGGG 16		IOVYSRHPPENGKPWI	16	7-22	9	
		INTKCYKLEHPVIGCG	91 /	85-100	9	

oble 3, continue

A PROTEIN SOURCE	PEPTIDE SECUENCE	LENGTH	POSITION	REFERENCE
451cin 0100	YKIMFYEDITRAKL	14	211-224	25
יי ופוכוף. אוני	IOTIKKNENIKEL	13	338-350	52
net A chain	DVGEYRAVTELGREDAEYWN	50	43-62	28
HIV 017	ERFAVNPGLLETSEGC	16	41-56	9
	DNYRGYSLGNWCAAKFESNFTO	23	20-42	v
U	EAL VROGE AKVAYVKPINT	20	101-120	9
70.	PIVONIOGOMVHQAIS	16	1-16	9
3	SAI SEGATPODLNIML	16	41-56	9
•	SFYILAHTETIDTETD	16	61-76	9
P1.8-	KMYFNLINTKCYKLEH	16	76-62	9

MALD-MS

TABLE 4 MST/HLA-DR2 BINDING PEPTIES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	2		
			18	DR2-3-57	2190.4	2189.0	
Pseudo HLA-A2	103-120	VGSDWRFLRGTHUTATUG	: 1	DR2-3-57	2133.3	2131.8	
	103-119	VGSDWRFLRGYHOYAYAD	- 2	082-3-56	2034.3	2040.4	
	104-120	GSDURFLRGYHQYAYDG	<u>.</u> ¥	DR2-3-56	1855.0	1858.5	
	103-117	VGSDURFLRGYHOYA	2 ;	082-3-56	1784.0	1786.3	
	103-116	VGSDURFLRGYHQY	£ ;	DR2-3-55	1755.3	1755.0*	
	104-117	GSDWRFLRGYHQYA	<u>.</u>	082-3-56	1698.2	1702.6	
	105-117	SDURFLRGYHQYA	<u>.</u>	082-3-70	2676.4	2675.0*	
Invariant Chain	96-119	LPKPPKPVSKMRMATPLLMGALPM	<b>3</b> 7	D82-3-70	2563.2	2562.0*	
(11)	911-76	PKPPKPVSKHRHATPLLHGALPM	3 8	DR2-3-70	2466.1	2465.0*	
	98-119	KPPKPVSKMRMATPLLMQALPM	7 (	082-3-66	2432.0	2437.0	
	97-118	PKPPKPVSKMRMATPLLMGALP	* *	20 C 200	2334.9	2340.0	
	98-118	KPPKPVSKMRMATPLLMGALP	7 %	DR2-3-70	2206.7	2207.0*	
	99-118	PPKPVSKMRMATPLLMGALP	0, 0	DR2-3-71	2070.5	2074.3	
	105-123	KHRHATPLLHQALPHGALP	<u>&gt;</u>	082-3-70	1732.2	1732.0*	
	105-119	KHRMATPLLHGALPH	c	DR2-3-65	2746.1	2746.6	
(MEI) Kinase-relate	59-81	EHHIFLGATNYIYVLNEEDLOKV	3	1			
Trasforming protein			11	DR2-3-71	2063.4	2074.3	
Guanylate-bind.	434-450	QELKNKYYQVPRKGIQA	<u> </u>	DR2-3-70	2248.5	2248.0*	
Mannose-bind. prot.	174-193	IONLIKEEAFLGITDEKTEG	2 ;	59-1-690	15055	15097	
HI A - DRZa & - chain	1-127	GDTRPRFL@ODKYECHFFNGTERVRFL	/21	082-3-66	17671	15013	
	1-126	GD TRPRFL QQDKYECHFFNGTERVRFL	971	DR2-3-70		~	
HLA-DR2b 8-chain	1-127	GDTRPRFLUGPKRECHFFNGTERVRFL	/2)	082-3-71		15009	
	1-126	<b>GDTRPRFLUQPKRECHFFNGTERVRFL</b>	971	082-3-39	2106.5	2114.	
	94-111	RVQPKVTVYPSKTQPLQH	ō Ř	DR2-3-39	1728.3	1730.6	
	94-108	RVOPKVIVYPSKIOP	2				

TABLE S WF-20/HLA-DR3 NATURALLY PROCESSED PEPTIDES

Pseudo HLA-A2         103-117         VGSDURFLRGYHQYA           Apolipoprotein         1276-1295         MFLKSDGRIKYTLNK           B-100 (Human)         1273-1291         IPDNLFLKSDGRIKYTLNK           1276-1291         NLFLKSDGRIKYTLNK           1276-1290         NLFLKSDGRIKYTLN           1276-1290         NLFLKSDGRIKYTLN           1207-1224         YANILLDRRVPQTDMTF           1-18         GOTRPRFLEYSTSECHFF           1-18         LPKPPKPVSKMRHATPLLHQALP           96-118         LPKPPKPVSKMRHATPLLHQALP				
1207-1224 YAN 1-18 G	15 KNSLK 20 19 16 15	DR3-2-63 DR3-2-63 DR3-2-60 DR3-2-60	1855.0 2352.9 2235.5 1910.2 1782.1	1863.9 2360.0 2245.1 1911.4 1785.9
1-18 6	11	DR3-2-63	2053.3	2059.1
	18 , 23 , 40ALP 22 , 21 , 40ALP 21	DR3-2-73 DR3-2-73 DR3-2-73 DR3-2-73	7 2545.2 2432.0 2334.9	2554.0 2441.4 2345.3

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## SUBSTITUTE SHEET

TABLE 6
PRIESS/HLA-DR4 MATURALLY PROCESSED PEPTIDES

							1
PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	Z	MASS SPEC	1
		SALVOSO ISOSTALISTA SALVOSO	21	DR4-2-45	2299.6	2304.0	
ig Kappa Chain	188-208	KHKVIACEVINGESSTVING	; 6	DR4-2-47	2212.5	2213.0	
C region (Human	188-207	KHKVYACEVIHOGESSPVIK	S &	DR4-2-43	1955.5	1952.1	
	189-206	HKVYACEVIHOGESSPVI	2 2	084-2-45	1883.1	1882.8	
	188-204	KHKVIACEVINGELSSP	: 4	DR4-2-45	1915.1	1922.5	
	187-203	EKHKVYACEVI HUGLSS	: ¥	DR4-2-54	1787.0	1787.0	
	188-203	KHKVYACEVIHUGLSS	ž <b>ž</b>	DR4-2-47	1755.0	1767.8	
	189-204	HKVYACEVIHGGLSSP	2 ¥	D84-2-43	1828.0	1822.8	
	187-202	EKHKVYACEVTHOGLS	ō Ř	084-2-51	1699.9	1708.3	
	188-202	KHKVYACEVTHOGLS	Ξ ¥	DR4-2-45	1657.8	1667.0	
	189-203	HKVYACEVTHOGLSS	2 2	DR4-2-51	1628.8	1632.6	
	187-200	EKHKVYACEVIHOG	<u>*</u>				
HLA-DR α-chain	182-198	APSPLPETTENVVCALG	17	DR4-2-43	1697.9	1700	70
			7	DR4-2-56	2470.6	2472.9	
HLA-A2	28-48	VODIGEVERUSDAASGAREER		DR4-2-59	2314.5	2319.3	
	28-47	VOD 1 DE VAFIO DE ANAMERICA DE LA COMPANSA DE LA CO	Ş Ç	DR4-2-54	2217.2	2218.7	
	28-46	VODIGEVERDSDAASGAAG	<u>.</u>	DR4-2-55	2256.4	2263.2	
	30-48	DIGFVRFUSDAASGRAEFR	<u>.</u>	084-2-56	2212.4	2211.5	
	31-49	IGFVKFUSDAASGKREFKA	. 4	DR4-2-55	1957.0	1963.1	
	58-82	VOU LEFVETUS DE SANS EN LE SANS E	17	DR4-2-56	1985.1	1987.5	
	51-47	MOCOAACOCICA	: <u>\$</u>	DR4-2-54	1758.9	1761.0	
	31-45		: 2	DR4-2-54	1343.4	1343.3	
	31-42	IDPVRFUSUAAS	!	1			

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28-50 31-52 28-48 28-47 28-46 31-48 31-48		LENGTH	FRACTION	3	MASS SPEC
				7 2236	7 756
	VDDTQFVRFDSDAASPRGEPRAP	82	DR4-2-20	1.555.1	
28-48 28-45 28-45 31-48 28-44	TOFVRFDSDAASPRGEPRAPW	22	DR4-2-54	2489.7	2491.5
28-47 28-45 31-48 28-44	VOOTOFVREDSDAASPRGEPR	21	DR4-2-54	2365.5	2368.1
28-45 28-45 31-48 28-44	VODTOEVREDSDAASPRGEP	20	DR4-2-56	2209.3	2211.5
28-45	MADIOEVEEDSDAASORGE	19	DR4-2-56	2112.2	2113.9
31-48		. <del>2</del>	DR4-2-56	1983.1	1987.5
28-44		82	DR4-2-52	2036.2	2041.5
<b>55-87</b>		17	DR4-2-55	1926.0	1931.7
		17	DR4-2-52	1897.9	1901.6
07-05		16	DR4-2-52	1596.7	1603.7
31-44	TOFVRFDSDAAS	12	DR4-2-54	1343.4	1343.3
			95-2-780	2374.6	2376.4
HLA-C 130-150	LRSWIAAD I AAGI I URKWEAA		087-2-20	1904.5	1908.7
129-145	DLRSWIAADIAAGIUR	- 3	08-2-20	1747.9	1752.3
129-144	OLR SWT AAD T AAG I 19	2			
129-143	DLRSWTAADTAAGIT	15	DR4-2-59	1619.7	7.2261
571.061	DI SSUTAADTAAQITOR	17	DR4-2-60	1834.9	1838.1
129-148	DL SSWTAADTAAQI TORKWE	50	DR4-2-66	2278.4	2284.6
731-131	YDHWEVKAINADDKSUT	17	DR4-2-70	2037.2	2039.6
	_			2035.3	
(Rat Homologue)	YDHNEVKAINADOKSW	16	DR4-2-70	1936.1	1937.7
				1934.2	
HLA-DR \beta-chain 1-14	GDTRPRFLEQVKHE	14	DR4-2-72	1711.9	
1G Heavy Chain 121-7	GVYFYLQWGRSTLVSVS (?)	(3)	DR4-2-6	٠-	~

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TABLE 7 MANN/HLA-DR7 NATURALLY PROCESSED PEPTIDES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	3	MSS SAEL
Pseudo HLA-A2	103-120	VGSDURFLRGYHQYAYDG VGSDURFLRGYHQYA	18	DR7-2-63	2190.4	1860
HLA-A29	234-253	RPAGDGTFGKHASVVVPSGQ RPAGDGTFGKHASVVV	20	DR7-2-66 DR7-2-63 DR7-3-66	2087.3 1717 2436	2092 1718 2440
	237-258 237-254 239-252 239-253	GDGTFQKNASVVVPSGGEGRT1 GDGTFQKNASVVVPSG GTFQKNASVVVPSG GTFQKNASVVVPSG GTFQKNASVVVPSGQ	22 15 15 23	DR7-2-66 DR7-2-66 DR7-2-66 DR7-2-66	1692.3 1462 1718 2603	1892 1465 1721 2606 2721
HLA-DR a-chain	58-78	GALANIAVDKANLEIMTKRSN	12	DR7-2-66		
Heat shock cognate 71 KD protein	38-54	TPSYVAFTDTERLIGDA TPSYVAFTDTERLIG	7 7 2	DR7-2-69 DR7-2-72 DR7-2-69	1856.0 1856.0 1669.8	1856.6 1857.0 1671.9
Invariant Chain	97-118	PKPPKPVSKMRMATPLLMGALP KPPKPVSKMRMATPLLMGALP	22	DR7-2-72 DR7-2-72	2432.0 2334.9	2436.6 2339.7
						MALD-MS

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MASS SPEC MALD-MS 1646.0 1848.4 1711.0 1595.0 1818.1 1799.9 1863.2 1699.8 1584.7 1632.9 1808.0 1789.0 3 DR8-3-63 DR8-3-78 FRACTION DR8-3-63 DR8-3-70 DR8-3-72 DR8-3-78 23.1/HLA-DRB MATURALLY PROCESSED PEPTIDES LENGTH TABLE 8 2 **5 7** 5 2 5 **EPFLYILGKSRVLEAD** TAFOY! IDNKGIDSO RSEEFLIAGKLOOGLL SEEFL I AGKLODGLL DVIVELLNHAGEHFG TAFQY! IDNKGIDS SEGLENCE 189-203 189-202 POSTT10M 261-275 101-117 102-117 38-53 Inhibitor 1 Prec. (Human) Transferrin (80V?) Metalloproteinase receptor (Hum?) PROTEIN SOURCE Cathepain S Celcitonin

TABLE Y HONZ/HLA-DR1 KATURALLY PROCESSED PEPTIDES

PROTEIN SOURCE	P0S1710M	SEQUENCE	LENGTH	FRACTION	₹	MASS SPEC
Pseudo HLA-A2	103-117	VGSDURFLRGYHQYA GSDURFLRGYHQYA	15 24	H2/DR1-1-64 H2/DR1-1-63	1855.0	1854.4
Invariant Chain (1i)	96-119 97-120 96-118 97-119 98-118	LPKPPKPVSKHRHATPLLHGALPH PKPPKPVSKHRHATPLLHGALPHG LPKPPKPVSKHRHATPLLHGALP KPPKPVSKHRHATPLLHGALPH KPPKPVSKHRHATPLLHGALPH KPPKPVSKHRHATPLLHGALP	% % & % & % & % & % & % & % & % & % & %	H2/DR1-1-77 H2/DR1-1-72 H2/DR1-1-73 H2/DR1-1-75 H2/DR1-1-75 H2/DR1-1-72	2676.4 2620.2 2545.2 2563.2 2466.1 2432.0 2334.9	2619.7 2619.7 2544.5 2562.3 2465.8 2431.7 2334.2
						ESI-MS

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INDIE 10
HANN SPLEEN DR4/DR11 NATURALLY PROCESSED PEPTIDES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	3	MASS SPEC
HLA-DR a-chain	133-156 136-156 136-155 136-151	SETVFLPREDHLFRKFHYLPFLPS VFLPREDHLFRKFHYLPFLPS VFLPREDHLFRKFHYLPFLP VFLPREDHLFRKFHYL	24 21 20 16	FFR.391-71 FFR.391-71 FFR.391-71	2659.1 2659.1 2572.0 2117.5	2982.5 2665.9 2579.6 2126.6
Calgranulin B	25-50 25-38	KLGHPDTLNGGEFKELVRKDLQNFLK KLGHPDTLNGGEFKELVRKDLQNF KLGHPDTLNQGEFK	56 24 14	FFR.391-71 FFR.391-71 FFR.391-71	3068.5 2827.2 1583.8	3073.0 2831.8 1591.2
	1					MALD-MS

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## CLAIMS

- 1. A purified preparation of a peptide comprising an amino acid sequence identical to that of a segment of a naturally-occurring human protein, said segment being 5 of 10 to 30 residues in length, inclusive, wherein said peptide binds to a human major histocompatibility complex (MHC) class II allotype.
- The preparation of claim 1, wherein said peptide binds to at least two distinct MHC class II 10 allotypes.
- The preparation of claim 1, wherein said human protein is HLA-A2, HLA-A29, HLA-Bw62, HLA-C, HLA-DRα, HLA-DRB, invariant chain (Ii), Ig kappa chain C region, Ig heavy chain, Na<sup>+</sup>/K<sup>+</sup> ATPase, transferrin, transferrin 15 receptor, calcitonin receptor, carboxypeptidase E, MET kinase-related transforming protein, guanylate-binding protein, mannose-binding protein, apolipoprotein B-100, cathepsin C, cathepsin S, metalloproteinase inhibitor 1 precursor, or heat shock cognate 71 kD protein.
- The preparation of claim 1, wherein said human 20 protein is an MHC class I or II molecule.
  - The preparation of claim 1, wherein said segment conforms to the following motif:
- at a first reference position (I) at or within 12 25 residues of the amino terminal residue of said segment, a positively charged residue or a large hydrophobic residue; and
  - at position I+5, a hydrogen bond donor residue.
- The preparation of claim 5, wherein said motif 30 comprises a hydrophobic residue at I+9.

- 7. The preparation of claim 6, wherein said motif additionally comprises, at position I+1 or I-1, a hydrophobic residue.
- 8. The preparation of claim 1, wherein said 5 segment comprises residues 29-40 or residues 1.06-115 of HLA-A2.
  - 9. The preparation of claim 1, wherein said segment comprises residues 107-116 of Ii.
    - 10. A therapeutic composition comprising
- 10 (a) a peptide comprising an amino acid sequence identical to that of a segment of a naturally-occurring human protein, said segment being of 10 to 30 residues in length, wherein said peptide binds to a human major histocompatibility complex (MHC) class II allotype; and

  (b) a pharmaceutically acceptable carrier.
  - (b) a pharmacourtain acceptance tanner.
- 11. A liposome containing a peptide comprising an amino acid sequence identical to that of a segment of a naturally-occurring human protein, said segment being of 10 to 30 residues in length, wherein said peptide binds to a human major histocompatibility complex (MHC) class II allotype.
- 12. An immune-stimulating complex (ISCOM) comprising a peptide comprising an amino acid sequence identical to that of a segment of a naturally-occurring human protein, said segment being of 10 to 30 residues in length, wherein said peptide binds to a human major histocompatibility complex (MHC) class II allotype.
  - 13. A method of inhibiting an immune response in a human patient, which method comprises contacting an

antigen-presenting cell (APC) of a patient with the therapeutic composition of claim 10.

- 14. A method of inhibiting an immune response in a human patient, which method comprises contacting an APC of a patient with the liposome of claim 11.
  - 15. A method of inhibiting an immune response in a human patient, which method comprises contacting an APC of a patient with the ISCOM of claim 12.
- 16. A nucleic acid encoding a polypeptide, said polypeptide comprising a first and a second amino acid sequence linked by a peptide bond, said first sequence being identical to that of a segment of a naturally-occurring human protein, which segment binds to a human MHC class II allotype and is of 10 to 30 residues in length; and said second sequence being a sequence which controls intracellular trafficking of a polypeptide to which it is attached ("trafficking sequence").
- 17. The nucleic acid of claim 16, wherein said trafficking sequence traffics said polypeptide to 20 endoplasmic reticulum (ER), a lysosome, or an endosome.
  - 18. The nucleic acid of claim 16, wherein said second sequence is substantially identical to the signal peptide of an MHC subunit.
- 19. The nucleic acid of claim 18, wherein said 25 subunit is an MHC class II  $\alpha$  or  $\beta$  subunit.
  - 20. The nucleic acid of claim 16, wherein said trafficking sequence is KDEL; KFERQ; QREFK; MAISGVPVLGFFIIAVLMSAQESWA; a pentapeptide comprising Q

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flanked on one side by four residues selected from K, R, D, E, F, I, V, and L; or a signal peptide.

- 21. A liposome or ISCOM comprising the nucleic acid of claim 16.
- 22. A nucleic acid encoding a polypeptide comprising a first and a second amino acid sequence linked by a peptide bond, said first sequence being identical to that of a segment of a naturally-occurring human protein, which segment binds to a human MHC class II allotype and is of 10 to 30 residues in length; and said second sequence being substantially identical to human Ii.
- 23. The nucleic acid of claim 22, wherein said polypeptide comprises a plurality of copies of said first sequence linked in tandem to said second sequence.
- 24. A nucleic acid molecule encoding a self peptide comprising an amino acid sequence identical to that of a segment of a naturally-occurring human protein, said segment being of 10 to 30 residues in length,
  20 wherein said self peptide binds to a human major histocompatibility complex (MHC) class II allotype, and wherein said nucleic acid molecule encodes less than the entire sequence of said protein.
- 25. The nucleic acid molecule of claim 24,
  25 wherein said molecule additionally encodes a peptide sequence which controls intracellular trafficking of a polypeptide to which it is attached ("trafficking sequence").

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- 26. The nucleic acid molecule of claim 25, wherein said molecule additionally encodes a second self peptide and a second trafficking sequence.
- 27. The nucleic acid molecule of claim 24,5 wherein said molecule additionally comprises expression control elements.
  - 28. The nucleic acid molecule of claim 24, wherein said molecule comprises plasmid or viral genomic sequence.
- 29. The nucleic acid molecule of claim 28, wherein said molecule is the genome of a non-replicative, non-virulent vaccinia virus, adenovirus, Epstein-Barr virus, or retrovirus.
- 30. A liposome or ISCOM comprising the nucleic 15 acid molecule of claim 24.
  - 31. A cell comprising the nucleic acid molecule of claim 27.
  - 32. The cell of claim 31, wherein said cell is a human B cell or APC.
- 20 33. The cell of claim 31, wherein said nucleic acid comprises genomic nucleic acid of a virus.
- 34. A method of making a peptide, which method comprises culturing the cell of claim 31 under conditions permitting expression of said peptide from said nucleic acid molecule.

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35. A method of inhibiting an immune response in a human patient, which method comprises introducing the nucleic acid of claim 24 into a plurality of APCs of said patient.

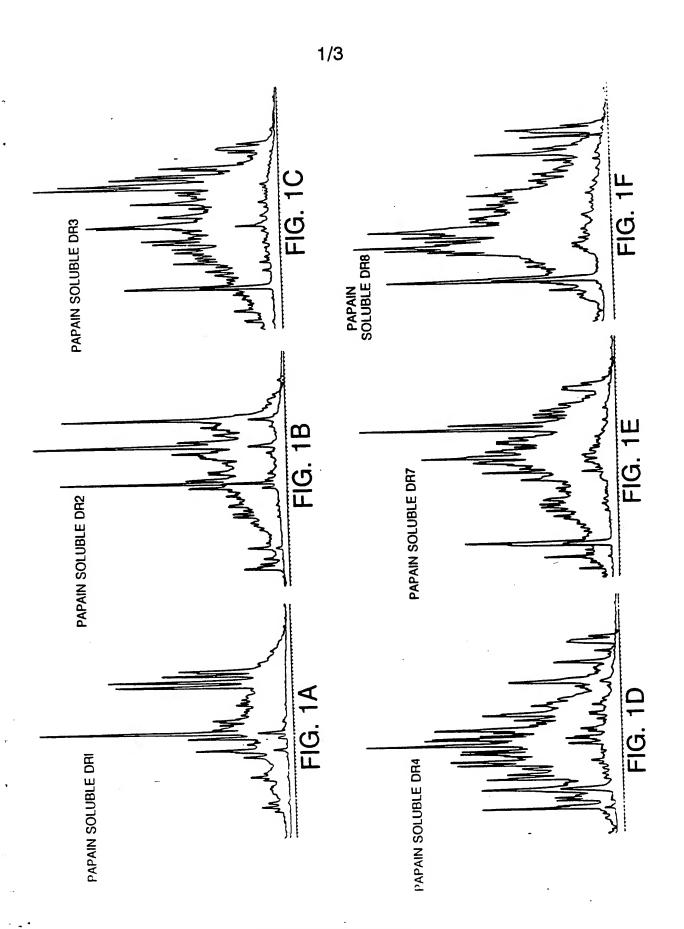
- 5 36. A therapeutic composition comprising the nucleic acid of claim 24 in a pharmaceutically acceptable carrier.
- 37. A method of inducing an immune response in a human patient, which method comprises introducing into an 10 APC of said patient a nucleic acid molecule encoding an immunogenic fragment of a protein of other than human origin, wherein said fragment binds to an MHC class I or II molecule.
- 38. The method of claim 37, wherein said protein 15 is of an infective agent which causes human or animal disease.
- 39. The method of claim 38, wherein said infective agent is human immunodeficiency virus (HIV), hepatitis B virus, measles virus, rubella virus, 20 influenza virus, rabies virus, Corynebacterium diphtheriae, Bordetella pertussis, Plasmodium spp., Schistosoma spp., Leishmania spp., Trypanasoma spp., or Mycobacterium lepre.
- 40. The preparation of claim 1, wherein said 25 segment consists essentially of a sequence set forth in any of Tables 1-10.
  - 41. A method of identifying a nonallelically restricted immunomodulating peptide, said method comprising:

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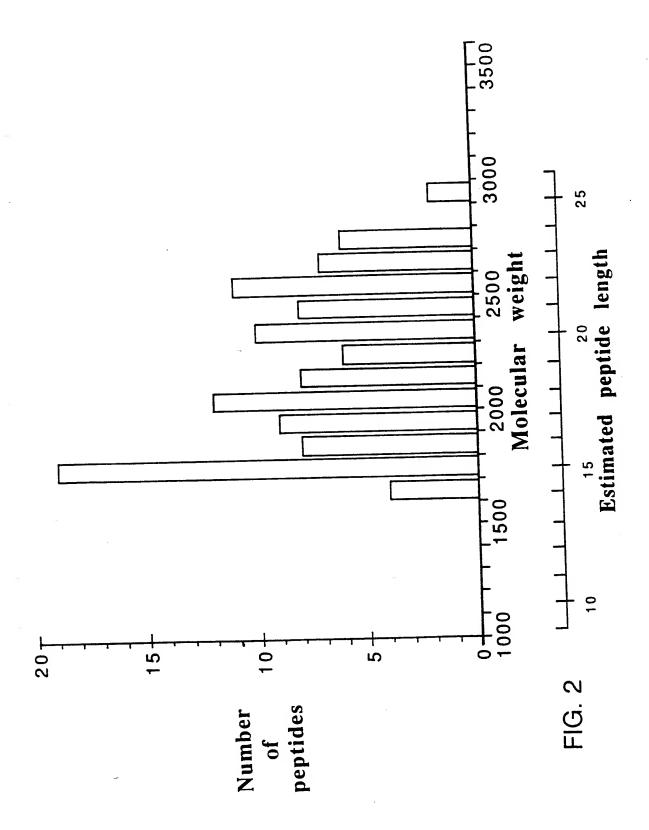
- (a) fractionating a mixture of peptides eluted from a first MHC class II allotype;
  - (b) identifying a self peptide from said mixture;
- (c) testing whether said self peptide binds to a 5 second MHC class II allotype, said binding being an indication that said self peptide is a nonallelically restricted immunomodulating peptide.
  - 42. A method of identifying a potential immunomodulating peptide, said method comprising:
- 10 (a) providing a cell expressing MHC class II molecules on its surface;
  - (b) introducing into said cell a nucleic acid encoding a candidate peptide;
- (c) determining whether the proportion of said 15 class II molecules which are bound to said candidate peptide is increased in the presence of said nucleic acid compared to the proportion bound in the absence of said nucleic acid, said increase being an indication that said candidate peptide is a potential immunomodulating 20 peptide.
  - 43. A method of identifying a potential immunomodulating peptide, said method comprising:
  - (a) providing a cell expressing MHC class II molecules on its surface;
- 25 (b) introducing into said cell a nucleic acid encoding a candidate peptide;
- (c) determining whether the level of MHC class II molecules on the surface of said cell is decreased in the presence of said nucleic acid compared to the level of said molecules in the absence of said nucleic acid, said decrease being an indication that said candidate peptide is a potential immunomodulating peptide.

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- 44. A method of identifying a nonallelically restricted immunostimulating peptide, said method comprising:
- (a) providing a cell bearing a first MHC class I 5 or class II allotype, said cell being infected with a pathogen;
  - (b) eluting a mixture of peptides bound to said cell's first MHC allotype;
- (c) identifying a candidate peptide from said 10 mixture, said candidate peptide being a fragment of a protein from said pathogen;
  - (d) testing whether said candidate peptide binds to a second MHC allotype, said binding being an indication that said candidate peptide is a
- 15 nonallelically restricted immunostimulating peptide.
  APPL3376



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ATG GCC ATA AGT GGA GTC CCT GTG CTA GGA TTT TTC ATC ATA GCT M A I S G V P V L G F F I I A

GTG CTG ATG AGC GCT CAG GAA TCA TGG GCT AAG ATG CGC ATG GCC V L M S A Q E S W A K M R M A

ACC CCG CTG CTG ATG CAG GCG CTG CCC ATG TAA

T P L L M O A L P M stop

FIG. 3A

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/IJS92/06692

IPCC): -AAAK 39/00, 39/385, 37/22; CONK 7/00; C12N 5/12 US CL: -Please See Eura sheet: Classification (IPC) or to both national classification and (IPC)  Minimum documentation searched (classification system followed by classification symbols) U.S.: 424/88, 450; 330/300; 536/27  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched once  Electronic data base consulted during the internstional search (name of data base and, where specially the search terms used)  The consultation searched other than minimum documentation to the extent that such documents are included in the fields searched once  Electronic data base consulted during the internstional search (name of data base and, where specially and the fields searched once  Electronic data base consulted during the internstional search (name of data base and, where specially and the fields searched once  Electronic data base consulted during the internstional search (name of data base and, where specially and the fields searched once  Electronic data base consulted during the internstional search (name of data base and, where specially and the fields searched once  Electronic data base consulted during the internstional search (name of data base and, where specially and the fields searched once  Electronic data base consulted during the internstional search (name of data trained and the search terms on the search once  Electronic data base consulted during the internstional search (name of data documents and the search once  Electronic data base consulted during the internstional files data base and, where special deciments and the search once  Electronic data base consulted files data base and the search once and	LIPCID: Asik 39/00, 39/385, 37/32: CONK 7/00; C12N 5/12 US CL. PRese See Extra Sheet. According to international Patent Classification (IPC) or to both national classification and IPC  B. FIELDS SCARCHED  Minimum documentation scarched (classification system followed by classification symbols) U.S.: 424/88, 430; 350/300; 536/27  Documentation scarched other than minimum documentation to the extent that such documents so included in the fields scarched none  Electronic data base consulted during the international search (name of data base and, where graphticable, search terms used) none  C. DOCUMENTS CONSIDERED TO BE RELEVANT  Category*  Ckation of document, with indication, where appropriate, of the relevant pastages  Relevant to claim No.  A US, A, 5,130,297 (Sharma, et al.) 14 July 1992. See entire document.  J. 210,1115  1-44  Critical reviews in immunology, vol. 11(5), issued 1992, J.C. Corgs, "Structura analysis of class II major histocompatibility complex proteins: "pages 305-335; see entire document.  J. 44  US, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  Low A, 100, A,	IPC(S)  ASIA 39/00, 39/38, 57/22; CDK 7/00; C12N 5/12  SC C. Pickas See Earts Since See Earts See Earts Since See Earts Since See Earts See Earts Since See Earts See	A. CL	ASSIFICATION OF SUBJECT MATTER		· · · · · · · · · · · · · · · · · · ·
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Marguides et al. "Engineering soluble smajor histocompatibility molecules: why and how", pages 101-116, see sentire document.  NATURE, vol. 348, issued 13 December 1990, F.M. Brodsky. 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DOCUMENTS CONSIDERED TO BE RELEVANT  Category*  Citation of document, with indication, where appropriate, of the relevant passages  Relevant to claim No.  X  US, A, 5,130,297 (Sharms, et al.) 14 July 1992. See entire document.  1.2.10,11-15  1.44  Critical reviews in immunology, vol. 11(5), issued 1992, 1.C. Corgg, "Structural rapidysis of class Il major histocompatibility complex proteins." pages 305-335, see entire document.  A  US, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.  1.44  Immunol. Res., vol. 6, issued 1987, D.H. Marguine et al. 'Engineering solitable immjor histocompatibility molecules: why and how', pages 101-116, see entire document.  A  NATURE, vol. 348, issued 13 December 1990, F.M. Brocksty. The invariant daring 1-44  Service', pages 581-582.  Further documents are listed in the continuation of Box C.  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